이학박사학위논문

Flexible and permanent strategies in foraging and antipredator behaviors of insects

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Abstract

Flexible and permanent strategies in foraging and antipredator behaviors of insects

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This dissertation presents my theoretical and empirical studies of flexibility focused on two topics of insect ethology. The first topic is the collective foraging strategy of the ants. Army ants were chosen as an example of extremely low behavioral flexibility in foraging; and on the other hand, carpenter ants were used to study mechanisms of extraordinarily high flexibility. My second interest is the behavioral control of aposematism, the phenomenon in which a defended prey animal gives a warning signal to predators who may recognize its advertised unprofitability.

In my first study, I investigated theoretical benefits potentially associated with the evolution of specialized foraging behavior performed by army ants. It is known that a typical colony of Neotropical army ants (subfamily Ecitoninae) regularly raids a large area around their bivouac by forming a narrow directional column that can reach up to one hundred meters in length. Then the raid is finished

and then relaunched 12–17 times, each time toward different orientations before the colony relocates to a new area. A hypothetical alternative to this foraging mode is raiding radially and symmetrically by expanding the search front in every direction like a circular bubble. Using an existing agent-based modeling software that simulates army ants' behavior, I compared the two possible modes of foraging in different food distributions. Regardless of the food patch abundance, the radial raiding was superior to the directional raiding when food patches had low quality, and the directional raiding was favored when the patches were rich. In terms of energy efficiency, the radial raiding was the better strategy in a wide range of conditions. In contrast, the directional raiding tended to yield more food per coverage area. Based on this model, I suggest that the directional raiding by army ants is an adaptation to the habitats with the abundance of high-quality food patches. This is the first theoretical argument for the adaptive value of the army ant behavioral syndrome which agrees with cumulated body of existing empirical measurements and descriptive models. This conclusion fits well with the known ecological conditions of army ants and their habitat.

In the second study, I conducted field experiments using wild colonies of carpenter ants (*Camponotus japonicus*). Unlike the army ants which obligatorily maintain their tight marching column, *C. japonicus* shows considerable variation in the coherence when foraging in a group. My investigation on this variability was centered on three sub-questions. First, I observed if higher group dispersion resulted in the lower chances of reaching the food source. As a dispersed group would cover a wider search area collectively, I believed that there would be some balancing disadvantage that can explain the coexistence of coherent and dispersed foraging behaviors. Second, I explored if there is any correlation between the group

coherence and the behavior of the scout, the key individual that first discovers the food source and subsequently summons multiple nestmates to the site. As the scouts of related species were known to have central control over their groups of recruits in various ways, I hypothesized that *C. japonicus* scouts would be also involved in the determination of group behavior. Third, I tested what would happen if I remove the scout from a group. I believed that the lack of scout pheromone would signal dispersion, as the scout seemed to be the source of coherence signals. After my analysis, I reached the following conclusions. First, higher group dispersion leads to lower success rates in correctly finding the food source. Second, the mobility of the scout in the pre-recruitment stage, her characteristic 'stroking' behavior during recruitment, and the dispersion of the recruited group were correlated to each other. Third, the simple lack of scout signal was not adequate to explain the observed variable reactions of the abandoned followers, and their response was linked to the pre-recruitment behaviors of the scout. I believe that *C. japonicus* possesses one of the most complicated recruitment strategy among the entire Formicidae, and the above findings rendered this species one of the best-understood ants in terms of mechanisms through which the flexible group foraging is controlled.

From the above two studies, I revealed each one of the ultimate and proximate mechanisms that maintain different levels of flexibility in ants' foraging strategy. My final topic, the behaviorally controlled aposematism, is a variant of aposematism in which the defended prey animal can choose to give different levels of anti-predatory warning signals depending on the circumstances. This 'switching' may occur in reaction to predators' approach (pre-attack signals) or attack (post-attack signals). The switchable aposematism has been relatively poorly studied, but

it could possess a variety of benefits. First, the switching could startle the predators (deimatism). Second, it could facilitate the aversive learning. Third, it could minimize the exposure or energetic expense, as the signal can be switched off. These potential benefits might offset the cost of developing, maintaining and utilizing the switchable traits. Here I focused on the third benefit of switchability, the cost-saving aspect, and developed an individual-based computer simulation of predators and prey. In 88 128 model runs, I observed the evolution of permanent, pre-attack, or post-attack aposematic signals of varying strength. I found that, in general, the pre-attack switchable aposematism may require moderate predator learning speed, high basal detectability, and moderate to high signal cost. On the other hand, the post-attack signals may arise under slow predator learning, low basal detectability, and high signal cost. When predator population turnover is fast, it may lead to the evolution of post-attack aposematic signals that are not conforming to the above tendency. I also suggest that the high switching cost may exert different pressure on the pre-attack and post-attack switchable strategies. To my knowledge, these are the first theoretical attempts to systematically explore the evolution of the switchable aposematism relative to permanent aposematism in defended prey.

These findings in common provided novel view into the proximate and ultimate mechanisms of behavioral flexibility found in insects. In addition, they exposed limitations of our understanding that called for future studies. First, I expect agonistic interactions with competitors or prey animals would considerably affect the collective foraging, aposematism, and food aversion, but I could not successfully provide universal background for such effects within the currently available datasets and literature in spite of many efforts. Second, the qualitative

difference of food items such as carbohydrate- or protein-richness is known to be pivotal in insect trophic ecology, but the scarcity of information and logistical limitations left me being unable to incorporate relevant enquiries into this dissertation. I predict that further discussions and experiments regarding these questions will reveal valuable information about the balance between the needs of resource exploitation and defense, which might greatly influence the behavioral flexibility of insects observed in the ecosystem.

Keywords : ant, foraging, column, scout, recruit, aposematism, switchability

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CHAPTER 1: INTRODUCTION

1. 1 Opening remarks

Behavioral flexibility is not a trait unique to animals with large and complicated brains. Many groups of insects are known for their ability to make temporary choices between different modes of behavior. For example, social insects can flexibly divide their colony tasks depending on the number (Garrison et al. 2018) and age (Calabi & Traniello 1989) of the available workers; various groups of pollinators may or may not constantly visit the same kind of flowers, depending on ecological conditions (Grüter & Ratnieks 2011); some intelligent insects can even bring their flexibility to a level seldom found in their phylum, as they are able to perform improved versions of observationally learned complex tasks such as tool use (Loukola et al. 2017).

In this dissertation, I focused on the examples that lie at the extremes of the spectrum of flexibility. As different species can show varying levels of constancy, the notion of flexibility should ideally be treated as a continuum. However, especially in the early stage of academic development concerning a specific topic, I believe we may benefit from cruder perspectives that study and categorize representative cases. For the sake of this simplicity, I will now focus on 'permanent' and 'flexible' strategies. As the name suggests, I define a permanent strategy as a behavior (or lack thereof) that an animal may alter remarkably little or none even after exposure to varying environments. A flexible strategy, on the other hand, is a behavioral adaptation that may change and revert according to ephemeral needs, without necessarily involving developmental plasticity.

This dissertation covers specific topics of interest to investigate the contrast between permanent and flexible strategies. The first interest is the collective foraging strategy of the ants. Foragers of many ant species can differentially communicate with their nestmates depending on the quality (Jackson & Chaline 2007; Horta-Vega et al. 2010), type (Portha et al. 2002; Cogni & Oliveira 2004; Le Breton & Fourcassie 2004) or quantity (Schatz et al. 1997; Robson & Traniello 1998; Mercier & Lenoir 1999), and the receivers of such information are known to show alternative responses to these signals. However, not every species have such flexibility, and those that have still manifest substantial variations in detailed mechanisms.

The second topic concerns the aposematism and behavior. Aposematism, named from Greek prefix apo- (away) and sema (sign), is the operation of deterrent signals given by toxic or otherwise defended prey animals (Ruxton et al. 2018). Red or yellow body colorations are the most commonly studied aposematic signals, but many other modes of warning exist; a bee's buzzing sound is acoustically aposematic (Jablonski et al. 2013), a ladybug's secretion has olfactory effects (Camarano et al. 2006), and a firefly larva glows intermittently to demotivate nocturnal predators (Underwood et al. 1997). These non-static signals are behaviorally controllable, but the evolution of such traits has been poorly studied.

In each of these two different topics, I attempted to choose two representative extremes as study subjects. This naturally suggested my inquiry to be divided into four separate parts: ant foraging strategies that are permanent and flexible, as well as aposematic systems that are similarly categorized. First, permanent collective foraging was studied via computer simulations of Neotropical army ants (subfamily Ecitoninae). Second, a local carpenter ant (*Camponotus*

japonicus) was chosen as its flexible counterpart and investigated via field experiments. The third was supposed to be about permanent aposematism, but as it is a topic that has been popularly studied since Darwin's time, this topic does not develop into a chapter here. The final topic was the flexibility of the aposematism, which was studied via abstract computer simulations that could be generalized over many taxa of relevant organisms. As apparent in this introduction, each of the study chapters was written as an independent project that fits the current needs and trends of each sub-discipline.

1. 2 Behavioral flexibility in ant foraging

The army ants are renowned for their enormous raid column that marches over hundreds of meters, containing millions of individuals (Couzin & Franks 2003). However, army ants are extremely inflexible in terms of foraging behavior. If a group of army ants is accidentally detached from the column, they will begin to follow each other blindly, rotating over the same place indefinitely (Das 2017). When caught in this infinite loop, they continue this behavior until they die of exhaustion, and do not attempt to break out to other modes of navigation. Similarly, many species of ants never form such a column; their foragers strictly hunt and gather alone, regardless of how large or strong their prey is (Fourcassie & Oliveira 2002). As they cannot summon their nestmates for help, they are not capable of assembling into foraging columns. However, other species of ants can perform both modes of behavior depending on the circumstances. Most ants can form a long, narrow marching column toward a specific food site if needs arise, but in other situations, they do navigate and search individually (Mercier & Lenoir 1999;

Liefke et al. 2001; Cogni & Oliveira 2004; Le Breton & Fourcassie 2004).

From this striking diversity in flexibility, two research questions were identified. First, the evident inefficiency of army ant behavioral syndrome called for a better evolutionary perspective. It has been believed that the army ants, though surprisingly successful, are caught at an evolutionary dead-end (Brady 2003). This argument mostly stems from their blindness and inability to break out of the formation, most vividly exemplified by the 'circular mill (Franks et al. 1991; Das 2017)' in which they follow each other in circles until death. The obligatory collective foraging seems to have indeed brought this clade into an "evolutionary stasis," and no known member of the taxon has escaped from the army ant behavioral syndrome. Its ecological origin, however, is not well understood. It is reasonable to assume that there had been evolutionary pressures that initially drove the ancestral army ants over this point of no return. In Chapter 2, alternative hypotheses regarding this topic are suggested and scrutinized in light of previous literature. Additionally, computer simulations were used to test potential benefits of food exploitation in various patch distributions.

The second question was centered on the behavioral flexibility of *Camponotus japonicus*, a soil-dwelling carpenter ant commonly found in East Asia. Foragers of this species occasionally recruit reinforcements with pheromone trails, but the details of this behavior are highly variable, to an extent rarely seen in other ants. A comparable level of flexibility had been previously described in a related genus but without much detail or quantitative analysis (Liefke et al. 2001). Particularly, the behavioral correlation of the key individual – the 'leader'- and the rest of the group was questioned, as past studies in other species left contradicting and limited conclusions (Mercier & Lenoir 1999; Portha et al. 2002). In Chapter 3,

the behavioral flexibility of *C. japonicus* foraging groups is described with a particular focus on the coherence of the formation.

1. 3 Behavioral flexibility in aposematism

Many prey animals change their appearance or emit other forms of signals when they encounter predators. This 'display' has long been a subject of evolutionary and cognitive biology (Edmunds 1972; Schlenoff 1985; Grandcolas & Desutter-Grandcolas 1998; Vallin et al. 2005; Bura et al. 2011; Umbers et al. 2015; Umbers & Mappes 2015). Such previous works have been mostly concentrated on the notion that the sudden display might startle the predators, providing the prey a window for escape. Nevertheless, this perspective considered little of the prey's actual defensive capabilities which could be irrelevant to how strongly the predator is surprised. Simple sensory exploitation without defensive measures might diminish in effect after repeated encounters, as the stimulus would be habituated and the predators may eventually learn the profitability of the prey (Bates & Fenton 1990; Olofsson et al. 2012). On the other hand, honest advertisements of toxins or other defenses could reach higher potency over time if the predatory guild learns the association between the signal and the unprofitability (Gelperin 1968; Kang et al. 2016b). Therefore, in spite of the superficial similarity, namely the displaying against approaching predators, the startle and the behaviorally controlled aposematism have completely different ecological and cognitive meanings from each other. Unfortunately, these two behaviors have been conventionally studied as one, mostly biased on the discussions pertinent only to startle mechanisms. In Chapter 4, a new name is given to the behavioral control of warning signals emitted by defended prey: the switchable aposematism. Afterward, its evolutionary

requirements and hypothetical origins are discussed with computer simulations.

CHAPTER 2. WHY DO ARMY ANTS, AND ONLY ARMY ANTS, FORAGE IN COLUMNS?

This chapter is a simulation study to reveal potential benefits of the army ant behavioural syndrome, which is characterized by raids in dense, narrow column formation. Army ants have very low flexibility in this behaviour, and therefore I chose them as an ideal topic to question ultimate evolutionary mechanisms behind permanent and flexible foraging strategies. Specifically, as this is quite inefficient search strategy, I focused on potential benefits that might compensate for the disadvantage.

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- ***Song W., Kim H., Lee S., Jablonski P. G. (2018) Directional raids by army ants as an adaption to patchily distributed food: a simulation model, Animal Cells and Systems, 22:4, 267-272, DOI: 10.1080/19768354.2018.1497708***

2. 1 Introduction

The army ants are specialized collective predators that always forage in large groups (Kronauer 2009). Their colony can form a swarm of many thousands of hunters, advancing in a column over a hundred meters long (Couzin & Franks 2003). In the 'nomadic phase,' they move their camp every day, but when a colony enters 'statary phase,' it launches multiple successive 'raids (Willson et al. 2011).' The raids occur about once a day, and they avoid the recently exploited direction (Willson et al. 2011). After depleting a region with about 14-17 raids, the colony relocates to a new area (Willson et al. 2011). Their nomadism depends on their skills to form a 'bivouac,' a huge ball of ants that temporarily shelter the young and the queen (Anderson et al. 2002). This set of behaviors has been evolutionarily conserved in more than 200 species of this clade over two continents (Brady 2003).

However, from the exploratory viewpoint, their columnar raid formation is an unusual choice. The search front is narrow (as short as one tenth of the column length; Couzin & Franks 2003), and only the minority of the foragers are exposed to the novel environment. The remaining majority run over the same path as their predecessors did, contributing almost nothing to the search. The successive raids will increase the final coverage area, but still it seems inferior to non-directed search patterns. For example, an *Eciton burchelli* raid can employ 200,000 individuals (Couzin & Franks 2003), and if they were to radiate uniformly from the colony in every direction, they could form an unbroken ring as large as 63,000 body-lengths in diameter. Even with minor workers, this would be nearly 200 meters wide, and it would not miss any single food item within the expanding 'bubble'. This would provide much better coverage than the column raiding does.

On the other hand, while being inferior in terms of exploration, the directional column raiding enables instant mass transportation after discovery. If the target food source is far away, the colony may save a considerable time by skipping the return trip of the discoverers and the dispatch trip of the recruited transporters. However, to justify having hundreds of thousands of potential transporters following the search front, the colony needs to ensure that the frontiers will find a very rich food patch. Otherwise, it could end up in a waste of time and energy for a very little gain. Therefore, the distribution of food should be a major parameter affecting the advantages of the directional column raiding.

To test the raiding performance under different food distributions, I chose a simulation software (Brown 2008) aimed at modelling *Eciton* species, the popularly studied new world army ants. I modified the program to enable comparison between the naturally occurring 'directional raiding' and the hypothetical 'radial raiding' strategies. I expected that the radial raiding would provide better coverage, but the directional raiding would yield more food in a certain range of food distributions.

2. 2 Methods

I used *AntSpace* 1.1 (Brown 2008), a *NetLogo* (Wilensky 1999) model of army ant raiding behavior. *AntSpace* combines many findings from the past (Deneubourg et al. 1989; Franks et al. 1991; Sole et al. 2000; Couzin & Franks 2003; Brown 2006) and faithfully simulates the army ant behavior. In *AntSpace* 1.1, the ants were assumed to move northward by default, and to stochastically choose their direction by comparing pheromone concentration between the north, the northwest and the

northeast grids. I modified the program to accept arbitrary raiding direction and resized the simulated world to 600 × 600 = 360,000 grids. The bivouac was repositioned to the center.

In order to simulate the foraging bout cycle, after 650 simulated time steps all outside foragers were called back to the bivouac. After extra 650 time steps in return phase, the pheromone deposits were all set to 0, and then the raid was re-launched. Fifteen bout cycles were simulated before recording the performance, to imitate *Eciton burchelli* statary phases that launch 14-17 raids before relocating (Willson et al. 2011).

In the directional raid mode, the direction of the new raid was randomly chosen within the half–circle located opposite to the previous raid direction (Fig. 1A). This was to simulate the actual ant behavior of avoiding the recently raided direction (Franks 1989; Willson et al. 2011). In the radial raid mode, each individual had its own raid direction randomly chosen before leaving the bivouac (**Fig. 2.1B**).

The food patch abundance values were 0.04%, 0.2% and 1%, and the patch quality values were 1, 10 and 100. In *AntSpace* 1.1, the food patch abundance means the proportion of food-loaded grids among all 360,000 grids, and the patch quality is the number of visits required to deplete the grid. This gave 3 × 3 = 9 food distribution conditions. The number of ants were set to 2000, which is similar to the number used in the previous simulations (Sole et al. 2000; Brown 2006). For all the other parameters *AntSpace* requires, I used empirically determined default parameter values provided with the software (Table 1).

The ‘coverage area’ of the colony was measured by the count of grids visited by an ant at least once. In order to represent collective energy use by the

colony, a variable 'total movement' was incremented by 1 every time an ant moved to another grid.

To measure the performance of the colony, the total 'food collected' was recorded. It was divided by the total movement or by the coverage area to demonstrate the different aspects of foraging efficiency. Another measurement, the proportion of the collected food to the maximum available amount, represented the ecological impact of the ants on the food resources.

2. 3 Results

For low-quality food patches (value 1 or 10) and regardless of the food patch abundance, the radially raiding ants collected 29-63% more food compared to the directionally raiding ants (**Fig. 2.2E, I**). However, for high-quality food patches (100), the directionally raiding ants collected more food overall (**Fig. 2.2A**). A similar general pattern could be seen in the two efficiency measurements (the second and third columns of **Fig. 2.2**). However, the two measurements differed in details. In terms of the movement efficiency, the food collected in one million collective movements, the radial raiding was the better strategy in general (**Fig 2.2B, F, J**). In a wide range of conditions the radial raiding outperformed or closely matched the directional raiding, often winning by margin of almost 90% (**Fig 2.2F, J**). Only in one condition (the middle plot of **Fig. 2.2B**), the directional raiding was 13% better.

In terms of the coverage efficiency, the food collected in one million explored grids, the directional raiding was a good strategy overall (**Fig. 2.2C, G,**

K). For low-quality food patches (i.e. when the patch quality was 1; **Fig. 2.2K**), the directional raiding was less efficient, but the gap was not greater than 11%. For higher-quality patches, the directional raiding performed better, and when the patch quality was 100, the margin was as large as 70-100% (**Fig. 2.2C**).

Food exploitation efficiency (proportion of food collected) was lower in conditions of high-quality food patches, regardless of food patch abundance (**Fig. 2.2D, H, L**). In conditions where radial raiding yielded more food, 34-59% of the total available food was collected (**Fig. 2.2H, L**). On the other hand, in conditions where directional raiding was superior, only 11-21% of the total food was collected (**Fig. 2.2D**).

2. 4 Discussion

Although the simulation showed that the directional raiding is generally coverage-efficient, this mode of foraging is not very energy-efficient (the second and third columns of **Fig. 2.2**). These trends are likely to arise when a large crowd of ants is concentrated in a small number of food patches. In this situation, most of the individuals are active in the already visited area rather than a new unexplored territory, leading to a more thorough search and the higher coverage efficiency. However, the movement efficiency may be negatively impacted by collisions between individuals due to the high density.

Why do the army ants raid directionally? I believe that the coverage efficiency is unlikely to be the ultimate reason, because it is difficult to find a selective pressure that may adaptively constrain the raid coverage. Neotropical army ants are the top predator of the ecosystem (O'Donnell et al. 2007) except

when they rarely encounter the anteaters (Willson et al. 2011), and unlike the Afrotropical Dorylus (Wilson 1971), inter-colonial conflicts are easily resolved without much mortality (Willson et al. 2011). They also have a set of behaviors specifically tuned to access difficult terrains, such as the 'living bridge (Graham et al. 2017; Reid et al. 2015)' or the 'pothole plug (Powell & Franks 2007),' implying that they gain benefit by expanding their activity range. Finally, they are nomadic species without permanent shelter, and they frequently relocate to a newer area (Kronauer 2009; Willson et al. 2011; Garnier & Kronauer 2017) suggesting again that they do not pursue smaller coverage.

Can selection in a foraging context explain why the army ants raid directionally? my model demonstrated that the directional column raiding was not a good foraging strategy to search for scattered small food sources. The model parameters were determined from the observation, so the natural selection could have optimized them for the directional raiding. In contrast, the radial raiding behavior in my simulation did not involve any further optimization to the new foraging regime. Only with the diversification of the initial departing directions, just one simple alteration of the model parameter, the colony gained substantial energetic reward in a wide range of test conditions. Compared to the radial raiding, the 15 directional raids were often inadequate to provide coverage over the full circle of range available to the colony, and left many food patches unexploited.

However, if the food patches were of very high quality, the directional raiding had advantages in various aspects of efficiency. Unlike the radial raiding, the directional raiding could maintain the density of the search front even after a considerably long expedition. This would allow fast and instant concentration of the workforce into a resourceful patch, draining it within a short time. After that,

the subsequent raids are unlikely to re-visit the depleted patch. On the other hand, in a radial raiding, it was difficult to recruit the remotely scattered foragers to the discovered patch. The discoverers could lay a pheromone trail back to the bivouac, but the information could not reach the majority of the outside foragers until they come back home. This bottlenecked the transition from exploring to transporting jobs.

The previous research on army ants support the adaptive value of directional raids in habitats with high food patch quality. Army ants, both the neotropical and the Afrotropical groups, are believed to have evolved from a common Gondwanan ancestral clade that preyed on social insect colonies (Brady 2003; Berghoff 2003; Brady et al. 2014), and numerous species still maintain the diet (Berghoff 2003; Ramirez & Cameron 2003; Powell & Clark 2004; Breton et al. 2007; Souza & Moura 2008; Kronauer 2009; Le Powell 2011; Dejean et al. 2014). Others have their diet diversified, but they also generally opt for large preys or rich litter patches (O'Donnell et al. 2005; Kaspari et al. 2011). A study reported that some army ant species generally cherry-pick higher quality patches, only skimming the most convenient 25% of the animal biomass and leave the rest intact (Kaspari et al. 2011). Interestingly, in my simulation, the conditions favorable to the directional raiding were identical to the conditions of less exhaustive exploitations (**Fig. 2.2D, H, L**). To sum up, the directional raiding is a trait closely related to highly rich resources that are not easily exhaustible, both in the real world and in my simulation.

Then, why the majority of other ant species that rely on rich food patches, e.g. the leafcutter and honeydew-harvesting ants, do not utilize the directional column raiding? In these species, a large number of reserve recruits waiting in the

nest compensates the downside of the undirected search (Jaffe & Deneubourg 1992). When a recruitment signal is given, the reserves follow the pheromone trail to the newly discovered food source, allowing massive and concentrated exploitation (Jaffe & Deneubourg, 1992; Shaffer et al. 2013). Most ant species have highly varied recruitment strategies based on this principle, implying the evolutionary flexibility and universal utility of this behavioral scheme (Hölldobler & Wilson 1990). In contrast, army ant foragers could not benefit from this recruitment scheme, because they leave the bivouac at a much faster rate and save less reserve in the colony (Deneubourg et al. 1989; Sole et al. 2000; Brown 2006). This extreme scout-reserve imbalance is probably for overcoming the specialized prey defense mechanisms (Kessler et al. 2016; Dejean & Corbara, 2014; Dejean et al. 2014).

The model does not include some other possible advantages of directional column raiding. First, the large number may allow the ants to overcome physical obstacles collectively by forming self-assembled bridges (Reid et al. 2015; Graham et al. 2017). Second, their large number and density might serve as a protection against predation or competitive aggression. Third, large and mobile prey e.g. living vertebrates could be quickly overwhelmed right after the discovery.

However, these additional benefits from directional raiding by a large number of individuals may not always be high. First, the self-assembled structures are costly commitments of many potentially active foragers, and their traffic enhancement is actually not very great (Powell & Franks 2007; Brunelle 2011). During colony migration, such bridges or rafts would be worth constructing because the queen, pupae, larvae and eggs need to be transported. However, in the foraging context it is difficult to think of a situation where a heavy investment in

traffic infrastructure is more important than increasing the coverage area. Second, as noted previously, the selective pressure from predation and competitive aggression is quite low for new world army ants (O'Donnell et al. 2007; Willson et al. 2011). Finally, although some species of army ants do hunt vertebrates (O'Donnell et al. 2005), most army ants primarily feed on social insect colonies (Berghoff 2003). Social insect colonies are immobile food sources and successfully exploitable with non-army-ant behaviors, e.g. by the termite-eating Matabele ants (Villet 1990) and the slave-making social parasites (Alloway 1979; Hasegawa & Yamaguchi 1994). Therefore, I excluded the aforementioned factors from the simulation and focused on the effect of the food distribution only.

In summary, this study illustrates that food distribution alone is sufficient to create ecological situations in which the natural selection may favor column raiding over the radial searching. The future studies should consider variations in different movement parameters as well as in the diet and the colony size to further investigate adaptive value of the raiding behavior.

Table 2.1 Default parameter values set in *AntSpace* 1.1 (Brown 2008), extracted from the past mathematical models (Deneubourg et al., 1989; Sole et al. 2000).

For detailed simulation algorithm which runs on these parameters, please see the publicly available code and information on *AntSpace* 1.1 (Brown 2008). For empirical basis and basic modelling principles underlying the choice of the parameters, please see Sole et al. (2000)

Parameter	Value	Explanation
maxPher-Return	540	threshold pheromone level at which returning ants refuse to deposit additionally
maxPher-Out	51	threshold pheromone level at which outbound ants refuse to deposit additionally
amtPherToRemove	0.005	amount of pheromone evaporated at each time step
amtPherToDrop	47	amount of pheromone deposited by outbound ants at each time step, given that returning ants deposit 10
emptyNodeweightOut	24	basal pheromone level outbound ants perceive from a grid without pheromone
emptyNodeweightIn	24	basal pheromone level returning ants perceive from a grid without pheromone
antsPerStep	10	number of ants that can simultaneously depart from the bivouac per one time step

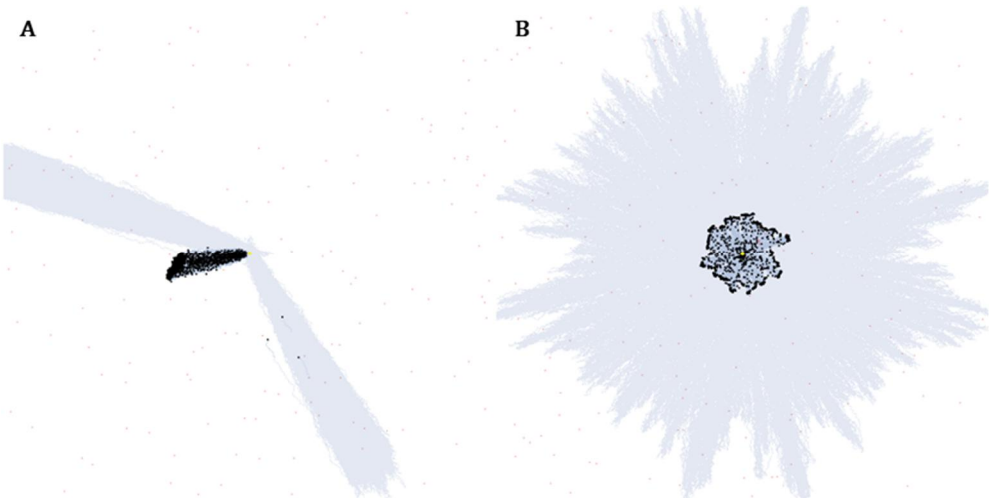

Figure 2.1 Simulated examples of directional and radial raids.

The beginning stages of both directional (A) and radial (B) raids are shown. In both examples, the colony (center) is launching the third raid out of 15 scheduled. The area covered by the previous raids is visualized with pale blue color.

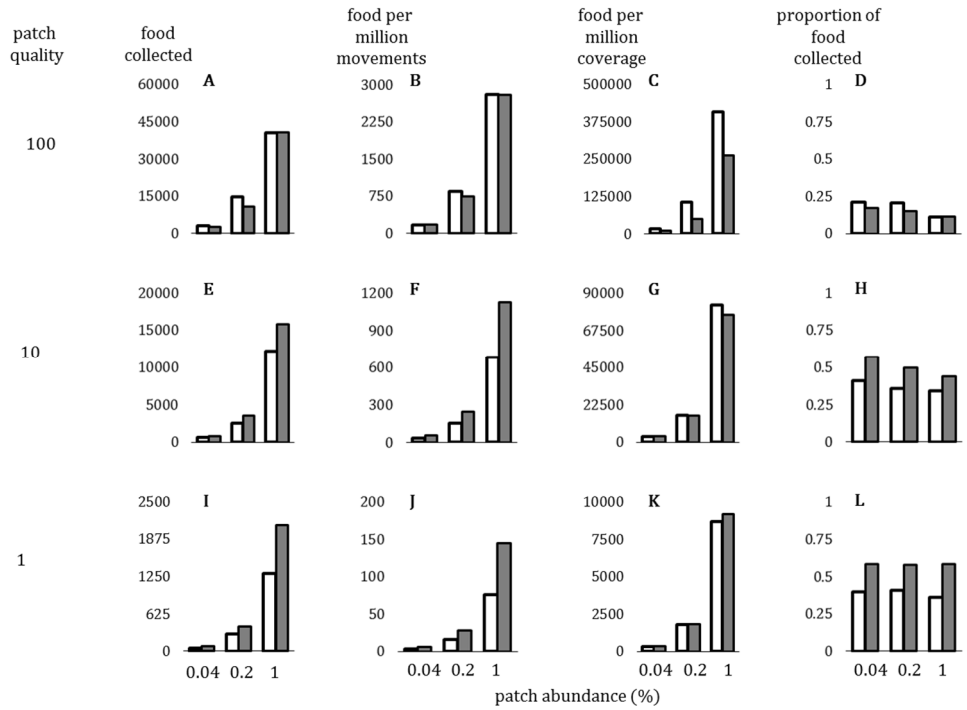

Figure 2.2 Colony foraging records after 15 raids are completed.

White, directional raiding; grey, radial raiding. First column (A, E, I), total number of food obtained. Second column (B, F, J), number of food obtained per one million collective movements. Third column (C, G, K), number of food obtained per one million grids collectively discovered. Fourth column (D, H, L), the collected proportion among the initially available amount. Subpanels are organized in three rows, according to the food patch quality set in the simulation. Each subpanel has horizontal axis for the abundance of food patches.

CHAPTER 3. COLUMNAR FORAGING WITH VARYING COHESIVENESS: BEHAVIOR OF *Camponotus japonicus*

In the previous chapter, I discussed the ultimate evolutionary mechanisms that could determine the flexibility of the ants' foraging behavior. The efficiency in food collection was suggested as a decisive factor, and I argued that the forager recruitment would shape the coherence of the foraging group. In this chapter, I delved into the proximate mechanisms of flexibility that could support different foraging strategies in ants. Especially, the question of behavioral diversity seen in recruitment attracted my attention. In order to explore the details of this flexibility, I chose a common carpenter ant species, *Camponotus japonicus*, as my study subject. This chapter covers my field experiments conducted on wild colonies of *C. japonicus*.

3. 1 Introduction

Many ant species rely on both centralized and distributed activities in collective foraging, and the balance between the two modes of behavior shows considerable variation. For example, a scout who discovered a novel food source often has full control over the path of the initial pheromone trail, which would then be followed by large numbers of recruits (Holldobler 1971; Traniello 1977; Debiseau et al. 1994; Liefke et al. 2001; Collignon & Detrain 2010). However, depending on the species and occasions, the cohesiveness of the foraging formation can be wildly different. Army ants are at one extreme, which forms a tight column with extremely high

forager density and minimal individual autonomy (Kronauer 2009; Couzin & Franks 2003). On the other extreme, tropical species of genus *Polyrhachis* have unusual and highly variable recruitment behavior that is so chaotic and autonomous that the researchers had to coin a new term, "leader-independent trail communication," to describe their difficult-to-classify foraging pattern (Liefke et al. 2001).

My study species, *Camponotus japonicus*, is a ground-dwelling carpenter ant species common in East Asia. According to my preliminary observations, foragers of this species occasionally recruit their nestmates with pheromone trails. After a scout discovers a food item, she may return to the nest, and then eventually reappear and lead a group of foragers toward the food source. When the scout becomes a group leader, she deposits pheromone on the way, and the other members of the group follow the trail. This set of behavior, called scout-recruit system, is widely adopted by social insects (Anderson 2001). However, I noticed that the recruits' fidelity to the trail was unusually variable; sometimes they formed a very tight column, while in other times they only shared a vague general direction and showed high individual autonomy. After several pilot experiments to reproduce each mode of behavior with different environmental setups, I formed several hypotheses regarding the coherence of the foraging column.

First, I hypothesized that the tighter groups would perform better in terms of correctly guiding the foragers to the food site, while being inferior in terms of collective search efficiency. If the following foragers stay close to the leader's pheromone trail, it will be less likely to miss the route and become stray. However, as subsequent individuals follow the pathways that have been trodden by other predecessors, the group as a whole will not be exposed to adequate search area

(Song et al. 2018).

Second, I believed that certain behaviors and conditions of the scout would be associated with the coherence of the foraging group. As the scout might gather information about the food site and the surroundings, it is possible that she might utilize the knowledge during recruitment. This belief is supported by findings in other species of the same subfamily Formicinae (Mercier & Lenoir 1999; Portha et al. 2002). Specifically, I focused on the following hypotheses and rationales. (1) If the scout spends more time in travel, she may subsequently lead a tightly packed foraging group. During the long travel, she might have gained more knowledge about the surroundings, and therefore the need to collectively explore the area will be lessened. (2) If the scout spends less time at the food and in the nest, she may subsequently lead a loose, scattered foraging group. If the scout spends little time in feeding on the food and sharing it to the nestmates, she might be less enthusiastic about the food quality, and therefore her group might be motivated to search for better alternatives. (3) If the scout is exposed to competitors near the food site, she may subsequently lead a loose, scattered foraging group. Competing species could have severed or displaced the food item while the scout is occupied in the recruitment trip, and therefore she may need to instruct the group to search for wider area when she comes back.

Third, I postulated that there might be behavioral components of the leader that is associated to the group coherence of the followers. It was very likely that the signal to mediate the coherence is chemical in nature. However, the leader's behavior while discharging different chemicals (or same chemicals in different quantity or spatial distribution) might be visibly identifiable (Holldobler 1971; Mercier & Lenoir 1999).

Fourth, I intended to test if the information transfer regarding the group coherence would occur in the early phase of the recruitment. From previous studies conducted on other members of the genus *Camponotus* (Holldobler 1971; Traniello 1977; Hillery & Fell 2000; Kohl et al. 2001; Kohl et al. 2003), it is known that the recruitment is occurring in two distinct phases; initially the recruits are invited to follow the trail, and then the trail gives orientation for the rest of the trip. If this is true for *C. japonicus* as well, it is reasonable to assume that the 'invitation stage' might have some predisposing effect on the trail-fidelity of the followers.

In order to answer these questions, I consulted previous literature which covered experiments in the related topics (Holldobler 1971; Sakamoto & Yamane 1997; Schultheiss et al. 2015), and identified common limitations among them. First, modern video processing technology was not available for these works, and the researchers had to rely on manually recordable variables that inevitably miss a large amount of valuable information. Another problem is that they mostly conducted experiment either in the natural foraging environment or in captivity. The natural foraging ground is already full of mature pheromone trails that obscure the details of their communication, and most foragers have some experience about the surroundings that they can often navigate without the help of the trails. With both social pheromone and individual memory not controlled, it is not possible to cleanly isolate the effect of the behavior in question. Captive colonies address this problem, but in turn introduces another complication; with their natural foraging and exploration forbidden, and provided with unnatural substances, their food transport behavior and dietary needs can be drastically and unpredictably altered. This problem is not well-documented in academic literature, but in my observation, it is a relatively common issue encountered by hobbyist community. Without first

establishing the behavioral integrity of captive *C. japonicus* colony, I would not accept conclusions drawn from indoor captivity especially when it is about foraging, navigation or communication ecology.

As a measure to avoid the issues outlined above, I used an artificially constructed arena newly presented to wild colonies. Therefore, the recruits did not have any prior knowledge about the environment, and there was no pheromone residue that could be an alternative information source. Therefore, the recruits needed to solely rely on the information learned during the trip, in addition to any pheromone trail which I could experimentally control and isolate. In this condition, the recruits could learn the new environment and follow the leader signals without unnecessary noise. I investigated movement characteristics associated with the coherence and dispersion of the foraging group under various conditions, and applied cutting-edge video tracking technology to extract precise data about their detailed movements. By answering the aforementioned questioned with these methodological improvements, this study provides novel information about the recruiter-recruit interaction in ants.

3. 2 Methods

Animals. Ten wild nests of *Camponotus japonicus* Mayr were chosen in Seoul National University campus, South Korea (37.4582048,126.9503465). Before the experiments, foragers from each nest were tested for mutual aggression to avoid using polydomous residences of a single colony. Experiments were conducted in front of active nest entrances targeting the outside foragers. I did not discriminate morphological sub-castes among the foraging workers, following my observation

that the polymorphic classes did not show conspicuous task division in the foraging context. I identified the leader of a group by observing from the side and looking for the characteristic behavior, dragging of the gaster tip on the ground. In total, 325 individuals were observed in 24 groups, 24 being leaders and 301 being recruits. My initial plan was to sample three groups per colony, totaling 30; due to logistical mistakes and ongoing construction in the study site, six of the planned trials were missing.

Test arena. 1-cm-thick white compressed polystyrene boards (trade name FoamBoard™) bought from a local stationery store were used for constructing an open-top box arena (**Figure 3.1**). The dimension of the internal space was 100cm(L) X 50cm(W) X 3cm(H). The box shape was achieved by taping pieces of boards with 3M Scotch™ tapes on the outer side, where the subject ants cannot reach. The surrounding walls were coated with polytetrafluoroethylene grease (trade name Teflon™), both inside and outside, to prevent unpermitted entry and exit. On one short-side wall, a 3-cm-wide opening was cut at the center, and the ants were allowed to use the entrance via a paper bridge of the same width. The floor and bridge were lined with non-fluorescent paper obtained from local stationery store, and the lining was changed after each trial to remove pheromone trails. To prevent the ants relocating for thermoregulatory purposes, the entire area was homogeneously shaded under a large, blue-colored umbrella. The test arena was not a fixed installation, and I promptly placed and removed it for every trial, making it a novel unexplored territory for the subjects.

Bait. At the opposing end to the entrance opening, a freshly prepared was placed.

In order to prevent the ants finding the bait via simple wall-follower algorithm, there was a 10-cm gap between the wall and the bait. The bait was a freshly cut late-instar mealworm that are firmly latched to a slide glass using bread wire ties. This fixture increased the weight of the bait setup, preventing the instant transport of the food to the nest. Therefore the scout had no choice but to recruit more nestmates if she decided to transport the item.

Nonvolatile arthropod-marking dye. White, skyblue, or yellow nontoxic kids' oil pastel stick (DONG-A Pencil Co. Ltd.) was ground and mixed with soybean oil (Ottogi Co. Ltd.) of 4X volume and heated below simmering. When cooled, the mixture had proper consistency so that it could be easily applied to the ant cuticle without running. I invented this paint because most locally available dyes contained volatile solvents that excite the subject and nearby ants unless dried completely. Acrylic paints were not usable as they induced food response from some of the colonies.

Treatment. In a non-rainy day of September 2015-2018, an outbound scout near the arena were displaced to the bait, making her the initial discoverer. While the scout was feeding on the bait, she was color-marked with the custom-made nonvolatile dye. Then I waited during the scout's solitary feeding, exploration, transportation and any other behaviors. When the scout began leading a group of recruits toward bait, I performed one among the two treatment options. In the leader-removed (LR) treatment, the leader was displaced to a separate container using a glass vial, away from the arena. In the leader-present (LP) treatment, the glass vial was placed as close to the leader as possible, and then removed after 10

seconds. In some of the LP treatments, the vial was placed over a follower and then removed with the individual. The dataset does not discriminate this difference; all LP experiments were analyzed together as long as the leader was not touched. Each session lasted for 90-120 minutes. Consecutive sessions on a given colony were performed after two- to seven-day interval, expecting the colony to adjust back to its natural surroundings and to become free of any directional bias potentially developed during the previous session.

Video analysis. Every trial was filmed from above and later analyzed with the object tracking software AnTracks 1.014. I observed the entire trial and afterwards verified and corrected the tracking result. The timeline was cropped to exclude the short period before the actual treatment is made. Also, based on my observation that the ants' behaviors begin to alter as they approach the far-side wall or the bait, my analysis on each trajectory included only up to the point of 75cm distance from the entrance-side wall. The trajectory of each individual was sampled 30 times per second, yielding x- and y-coordinates.

Coordinate system. The filmed video was rotated and transformed to conform to a consistent coordinate system. In the x-y 2-dimensional plane, the experimental arena stretched from x=0 to x=100 and y=-25 to y=25, units being centimeters. The ants entered the arena through the entrance which was located at (0, 0). The bait was located at (90, 0). The shortest route from the entrance to bait was therefore a horizontal straight line (y=0 line).

Pre-recruitment variables. As the scout had to first return to the nest, recruit the

foragers, and then come back to the arena, I could gather many different variables before the recruitment starts. First, the scout made varying **number of trips** between the bait and the nest before she finally decides to recruit. Similarly, it took varied time until recruitment, and it was divided by **number of trips** to give the variable **time per trip**, measured in seconds. The time until recruitment could be divided into two portions; the 'immobile time', the time spent at food or in the nest, and the 'mobile time,' the time spent during the movement between the food and the nest. The proportion of mobile time over total time was defined as the variable **scout mobility**.

Finally, some interspecific competitors (mostly *Formica japonica* and *Vespula flaviceps*) were naturally present on the habitat, and some of them entered the arena and interacted with the scout. The presence of such competitors was recorded every 30 seconds, and if multiple of them were present, the recording was also multiplied accordingly. By adding up all such records, I generated the 'competitor presence ratio,' which was the competitor-present time divided by the total time. I also created 'competitor interaction ratio,' which is equivalent to the former but recording only the aggressive interaction instead of mere presence. As these two competition-indices are inherently correlated, I extracted the first principal component of these two variables, and named it the **competition component**.

Recruiting leader variables. When the scout begins to lead a group, she was deemed a leader and her movement was analyzed in more detail. Specifically, the **x-** and **y-speed** was calculated for each frame, and then averaged to give **mean x-speed** and **mean y-speed**. The coefficient of variation of each speed was also used

as a variable, **x-** or **y-speed variation**. The difference between the leader's minimum and maximum y-coordinate was defined as **y-range**. As these five variables were inherently correlated to one another, I extracted principal components (PCs) from them. In the statistical analysis, the resulting five PCs were used instead of the raw variables. Among the recruiting leader PCs, PC5 had a strongly dichotomous distribution supported by the Hartigaan's dip test; so in some analyses I categorized it to a binary categorical variable, renaming it the **leader category**.

Recruited follower variables. In the leader-present (LP) dataset, it was possible to calculate the distance to the leader trajectory. As the group entered the arena through the bridge, which forced them to move along the straight y=0 line, I assumed that they had directional bias to generally continue the trend. In times of uncertainty, they may naturally try to move along that direction to extrapolate (as indicated in Schultheiss et al. (2015)). Therefore, I only measured y-axis distance to the leader trajectory in order to isolate the 'deliberate' deviation from the trail. The maximum y-axis distance from the leader trajectory was used as the variable **follower dispersion**. Since some analyses called for a group variable, **follower dispersions** of all individuals in the group were averaged to yield **mean follower dispersion**.

In the leader-removed (LR) dataset, the leaders were not present, so the variable **follower dispersion** was not obtainable. Instead, the abandoned followers could choose to give up and return to home, or to advance on their own without the leader. In order to represent this variation, I measured the **maximum x coordinate** of the abandoned followers. Later, this variable turned out to be bimodally

distributed. Therefore, I made a binary-categorized version of this variable, **advancement after leader loss**. The category boundary was obtained from hierarchical clustering with Ward-D method.

In both of the LR and LP datasets, a follower may eventually arrive at the food site or not. This was coded as a binary variable **food-reach success**. As the followers recruited by the same leader are expected to have common tendencies, the analysis had to account for this nested structure. As single experiment session tested a single leader, the e**xperiment ID** served as the random-effect variable when needed.

Group variables. In some analyses, it was necessary to create some variables that can only be defined for the whole group. In addition to the **mean follower dispersion** introduced above, the collective search efficiency was also measured. After dividing the entire arena into 2.5 × 2.5 cm grids, the number of unique grid squares that were visited at least once by any member of the group was counted, and then divided by the number of individuals in the group. The **search efficiency** was defined as the number of unique grids visited per individual. As this value is expected to naturally decrease as the group size increases, the **number of followers** was also measured to account for this effect.

Statistical analysis. All analyses have been conducted with the statistical software R 3.6.1. (Generalized) linear mixed models were fitted with the *lme4* package. Model selection and averaging was performed by *MuMIn* package. To generate predictions from an averaged model, *AICcmodavg* package was used. Hartigaan's dip test for unimodality was performed with *diptest* package. In the subsections

below, I outline variables and specific methods used for each part.

Follower dispersion may be advantageous in search but not in faithful leading. A generalized linear mixed model (GLMM) with logit link was fitted with the binary response variable **food-reach success**, the fixed-effect predictor variable **follower dispersion**, and the random-intercept predictor variable **experiment ID**. In all mixed-effect models covered in this chapter the random effect structure was constrained to intercept-only, in order to avoid convergence problems associated with small-size groups (Grueber et al. 2011).

Afterward, a weighted linear model (LM) was fitted with the response variable **search efficiency** and the predictor variables **mean follower dispersion** and **number of followers**. As the model was fitted on a group variable whose accuracy is dependent on the group size, the **number of followers** was supplied as the weight vector.

Pre-recruitment conditions of the scout may predict the follower dispersion. A linear mixed model (LMM) was fitted with the response variable **follower dispersion**, the fixed-effect predictor variables **scout mobility, number of trips, time per trip**, and **competition component**, and the random-intercept predictor variable **experiment ID**. In all multiple-regression models used in this chapter, no interaction terms were included in order to avoid convergence problems associated with small-size samples, and the predictor variables were all scaled and centered.

The above model then served as a global model for automated model selection by corrected Akaike information criterion (AICc). Afterward, I selected the sub-models from the highest AICc until the cumulative sum of Akaike weight reaches 0.95. The selected sub-models were then averaged to yield the final model. In model averaging, dropped parameters in sub-models were assumed to have 0

coefficients ("full averaging").

A component of leader behavior may predict the follower dispersion. A linear mixed model was fitted and averaged in an identical procedure to the above, with a different set of fixed-effect predictor variables: PC1 through PC5 extracted from the recruiting leader behavior.

The follower dispersion may be decided in the early phase of the recruitment. First, a model was built to simulate leader category of the leader-removed (LR) dataset. The leader-present (LP) dataset which has the leader behavior components served as the 'train' set. A linear model (LM) was built with the response variable **leader category**, and the predictor variables **number of trips, time per trip, scout mobility**, and **competition component**. It was supplied as a global model to the selection and averaging procedure identical to the above.

Subsequently, the averaged model was applied to the LR dataset to generate the **predicted leader category**. Using the predictions as the new fixed-effect predictor, a generalized linear mixed model was fitted with logit link and the following set of variables. The response variable was the followers' **advancement after leader loss**, and the random-intercept predictor variable was **experiment ID**.

In order to provide a perspective of the LR dataset compared to the LP, and to replicate the previous studies (Holldobler 1971; Schultheiss et al. 2015) in my study species, I also measured the **food-reach success** of the abandoned followers after leader loss. It was modeled by the same approach as the above, with a different fixed-effect predictor **leader presence**.

3. 3 Results

As AIC-based approaches are advised to be detached from the notion of significance (Grueber et al. 2011) and the general problem of null-hypothesis significance testing (NHST) is widely recognized (Wasserstein & Assoc 2016), hereby most results are reported with descriptive languages, and significance is only mentioned when NHST context is clearly needed (such as Hartigaan's dip test). In other cases, p-values are reported and annotated in tables for convenience, but they are not regarded as a fixed criteria for accepting or rejecting hypotheses.

Descriptive statistics. Summary of variables used in the analysis is given in **Table 3.1A** (leader-present dataset) **and 3.1B** (leader-removed dataset).

Follower dispersion predicted food-reach failures. In the leader-present (LP) dataset, the **follower dispersion** is very likely a predictor of lower **food-reach success** (**Table 3.2; Figure 3.2**).

Follower dispersion predicted higher search efficiency. In the LP dataset, the **follower dispersion** is likely a positive predictor of higher value of collective **search efficiency** (**Table 3.3; Figure 3.3**).

Pre-recruitment scout mobility predicted recruited follower dispersion. In the LP dataset, the averaged model (**Table 3.4**) indicated that the **scout mobility** is potentially a predictor of higher **follower dispersion (Figure 3.4)**. However, **number of trips**, **time per trip**, and **competition component** had little evidence for treating them as important predictors.

Principal components of the leaders' recruiting behavior. In the LP dataset, the leaders' movement variables were restructured into five principal components (**Table 3.5**). I assigned alias for each of them for easier recognition. Biplots showing the composition of each component is given in **Figure 3.5**. The variance of leader movement was mostly explained by first four components (**Table 3.6; Figure 3.5D**). However, I decided to drop out no component based on the following two reasons. First, the pheromone-laying behavior could be affected by extremely micro-scale movements. Second, thanks to the high-quality video tracking provided by *Antracks*, I was confident that the measurement error would be negligible, so such micro-scale difference would be faithfully captured by principal components, as shown by **Figure 3.6**.

Recruiting leader PC5, the "stroking" component, predicted lower follower dispersion. In the LP dataset, model averaging indicated that the leader PC5 ("stroking" component) could likely be an important predictor of **follower dispersion** (**Table 3.7; Figure 3.7**). PC1 through PC4 had very little evidence for association with the follower dispersion.

The distribution of recruiting leader PC5 was not unimodal. As apparent in **Figure 3.7**, the leaders showed dichotomous distribution along "stroking" component. The dip test for unimodality gave Hartigan's dip statistic (HDS) 0.12509 (p=0.0408 *), indicating that the data distribution is significantly multimodal. Therefore, I categorized this variable and renamed it **leader category**. Leaders with high PC5 will now be deemed "stoking leaders," and those with low

PC5 will be called the “continuous leaders.”

Leader absence predicted food-reach failures. Table 3.8 and **Figure 3.8** show that the **leader presence** was highly likely a positive predictor of **food-reach success**.

The distribution of maximum x-coordinates of abandoned followers was not unimodal. In the leader-removed (LR) dataset, the **maximum x-coordinates** of the followers had bimodal distribution (**Figure 3.9**). Hartigan’s dip test confirmed that the distribution is significantly multimodal (HDS=0.106, p=<2.2e-16 ***). Hierarchical clustering identified the cluster boundary at 72 centimeters, and therefore I used that criterion to categorize this variable. I named the categorized variable **advancement after leader loss**.

Model-predicted leader category predicted the followers’ advancing after leader loss. In LR dataset**,** the **leader category** was predicted by the model (**Table 3.9**) and then suggested as one possible variable to predict the advancing followers (**Table 3.10**; **Figure 3.10**)

3. 4 Discussion

The scout mobility. In this study, I found that the pre-recruitment scout mobility was associated to the higher follower dispersion in the recruiting stage. High scout mobility could be arising from two reasons; first, the scout might be spending more time for moving, and second, the scout might be spending less time at food or nest.

It would be interesting if I could attribute the variation of the scout mobility to one of these two sources. However, my observation indicated that both factors might be playing a role, and during my analysis I found that it was not easy to reduce the complexity of this variable.

Unlike the recruitment behavior, information about the scout ants' pre-recruitment behavior is relatively scarce. Researches have been measuring time until recruitments and left descriptions about scout behaviors (Simola et al. 2016; Gruter et al. 2018; Zephirin et al. 2019), but these were not investigated further to find any subsequent recruit behavior. Some researchers characterized the foraging time by dividing them into two tasks, travelling and searching (Crist & MacMahon 1991), but the distinction between the recruiter and recruits were unclear. In a study of a Ponerine species, the time of prey-contact was associated to the recruitment, following a question relatively similar to mine; but as their study species was very fast-moving and reacts quickly to food discovery (less than a second of prey contact was not rare), the findings were not applicable to my study species, *C. japonicus*.

However, there are still some studies highly relevant to this variable. Scouts of *Polyrhachis labriosa*, a member of genus *Polyrhachis* that is closely related to my study genus *Camponotus,* spent more time in feeding if the food source was small in volume while less motivated to recruit (Mercier & Lenoir 1999). This study suggested that if the food source is not worth recruiting, the scout would attempt to transport it by herself instead of summoning more foragers. In this scenario, the lower scout mobility represented the lower recruit excitation, and therefore the followers would not choose to follow the leader very strongly.

In another study on *Lasius niger*, a member of the subfamily Formicinae,

reported that the type of food could alter both scout and recruit behavior. When a prey animal (naturally ephemeral food source) was presented instead of sucrose solution (naturally static food source), both the scout and the recruits showed wider search range (Portha et al. 2002). If *C. japonicus* behavior is in line with this theory, then the scout mobility would associate with the perceived stability of the food source, and therefore higher recruitment motivation, and presumably stronger follower fidelity to the leader trace.

As my result show that the more mobile scouts are associated to more scattered followers, I believe that the explanation for *L. niger* fits better to *C. japonicus*. Adopting this idea, however, leaves two questions. First, in my study the food source was always animal protein (a mealworm) and the quality and mobility of the food item was controlled. Why should there be such great variation in scout mobility and follower dispersion? Second, I observed in the field that recruitments are normally associated with proteins, not sugars. If the perceived stability of the food played a role in follower dispersion, wouldn't the expected pattern be the opposite, like in *L. niger*?

The simplest hypothetical explanation for these two problems is the scout personality. If some scouts are innately more explorative due to developmental or age reasons (a pattern demonstrated in *Pheidole dentata* (Muscedere et al. 2012)), and if such tendency is transferrable to the followers via stroking behavior, then the observed variation would become quite explicable. In order to test this hypothesis, repeated measurements from multiple leaders from a single colony would be required.

The stroking behavior and the leader category. It is interesting to find the

bimodality of the leader behavior, because authors of the related previous works (Mercier & Lenoir 1999; Le Breton & Fourcassie 2004) did not report such pattern in their results. Especially in the earlier study with *P. labriosa*, the researchers actually counted the number of stops made by the scout, assuming that these are the pheromone-discharging moments. Neither the detailed behavioral descriptions about members of *Camponotus* (Holldobler 1971; Traniello 1977; Sakamoto & Yamane 1997; Schultheiss et al. 2015) nor *Polyrhachis* (Mercier & Lenoir 1999; Liefke et al. 2001) have any mention about these two types of leaders. It could be argued that the dichotomy reported in this study is an artefact of small sample size, but from my unreported pilot experiments and field observations, I strongly believe that there is indeed a distinction between these two behavioral categories.

I speculate that this categorization might be only present in *C. japonicus* among the East Asian members of tribe Camponotini. As the common name 'carpenter ant' suggests, species of Camponotini nest mostly in wooden materials; the ground-dwelling *C. japonicus* is one of the rare exceptions. For example, according to a phylogenetic analysis conducted on common Eurasian formicines (Sameshima et al. 1999), *C. japonicus* was deeply embedded in a clade of 10 wood-preferring species, including the almost indistinguishable sister species *C. vagus*. This suggests that *C. japonicus* has recently underwent drastic change in their habitat in their evolutionary history. As some researchers argue that the arboreal and ground environments have different optimal recruitment strategies (Zephirin et al. 2019), it is plausible that the *C. japonicus* might have behavioral remnants of their arboreal ancestry that are uncommon in other ground-dwellers. If *C. japonicus* had two behavioral syndromes that could be executed in different contexts, it would provide a survival advantage in various environments. Detailed behavioral investigation of

its close relatives, especially *C. vagus* found in Europe and West Asia, would be necessary to test this hypothesis.

Possible mechanisms of dispersed recruitment. In this study, I found that the "continuous" class of leaders are more likely to recruit their followers in dispersed manner. The large distance between the leader trajectory and the dispersed follower trajectory would make trail-based communication substantially difficult. How are these leaders able to communicate the general direction of the food site to the naïve recruits, while allowing their free and unorganized movements?

Volatile, far-reaching pheromones could be the possible answer, but this idea lacks support from previous literature. An alternative explanation is that the scout leaves small amount of long-lasting trail pheromones scattered around the arena before beginning recruitment. These chemical cues would then be used by the recruits afterwards, working alongside the conventional group recruitments. This system has been documented in several species of *Polyrhachis* (Liefke et al. 2001). Another possibility is that the scout gives differential amount of excitation signal and the orientation signal; For *C. socius*, *C. pennsylvanicus* and several other members of *Camponotus*, it is well established that the excitation signal (poison gland material) can work independently from the trail markings (hindgut material) (Holldobler 1971; Traniello 1977; Kohl et al. 2001; Renyard et al. 2019). Although there is no empirical evidence that the recruiter can control the proportion of the two substances, it is very plausible to interpret the dispersed recruitment as a less orientating and more excitatory form of recruitment. I believe that differential recruitment observed in *Gnamptogenys modelleri* (Cogni & Oliveira 2004) might be utilizing a mechanism similar to what is suggested above. However, considering

the extremely far phylogenetic distance between the study species, one should proceed with caution before combining the findings above. Another complication for this hypothesis is my experience with *C. japonicus* response to formic acid, the major poison gland material that mediates recruiting in many related species (Traniello 1977; Kohl et al. 2001; Renyard et al. 2019). According to my preliminary observations, exposure to formic acid did not facilitate recruitments in *C. japonicus*, contradicting the previous reports about its relatives. If future research involves testing this hypothesis, deployment of highly advanced biochemistry techniques would be necessary to clear up the confusion.

Bimodal behaviors in abandoned followers. After leader loss, recruits of other *Camponotus* species generally failed to navigate unless given prior information (Holldobler 1971; Schultheiss et al. 2015). In my experiment, the test arena was small and only had one exit, and therefore all recruits could return to the nest or reach the food eventually. Therefore, their tendency of advancing was measured as a key variable instead of the final outcome. I demonstrated that the abandoned recruits are divided into two indisputably clear behavioral classes, those advance beyond x=62cm mark and those do not. A separation similar to this has been observed in *C. consobrinus* (Schultheiss et al. 2015), but the followers' prior experience level was not controlled, and the leaders were removed after long segments of trip that there would be enough information to directionally extrapolate. Therefore, the categorization observed in my experiment should be treated qualitatively different; there is no previous literature related to how other species' followers would separate under conditions similar to my leader-removal treatment. In addition to that, I established that the proportion between the advancing and non-advancing followers is related to the pre-recruitment behavior

of the removed leader. As this association is a complete novelty, it was difficult to find any previous discussion relevant to interpreting this result. Still, I was able to formulate three new hypotheses, each of them at least partially supported by literature.

First, there is variation among the foragers regarding how strongly they are attracted to pheromone trails, possibly due to different age (Muscedere et al. 2012). Both types of foragers are recruited by both types of leaders, but the "stroking" leaders tend to recruit individuals with stronger trail-following tendency, while the "continuous" leaders do not. When the leader is lost, those two types of recruits show difference in their explorative behavior. Recruits with stronger trail-following tendency would then refuse to leave the suddenly truncated trail after leader removal.

Second, at the beginning of the recruitment, the leader provides information about how intensively the recruits should follow the trail. There are a number of species known to have pheromone trails but never actually followed unless specific invitation behavior is performed by the leader (Traniello 1977; Le Breton & Fourcassie 2004). In this scenario, different leader categories might differ in how they engage in this invitation behavior.

Third, it is the characteristic of the trail itself that conveys information about how faithfully the recruits should walk along it. In this case, it might be the simple concentration or continuity of the trail (Jackson & Chaline 2007), or it might be some qualitative difference involving mixture of orientation and excitation materials (Holldobler 1971; Traniello 1977; Kohl et al. 2001; Renyard et al. 2019).

I regard that all of the tree hypotheses seem quite plausible, but at the same time,

all of them carry little weight as they did not cover the leader-loss situation. Future works to identify the underlying mechanism should include consideration for all three possibilities.

Concluding remarks.

The main contribution of this work is the discovery of association between pre-recruitment scout behavior, the scout's leading behavior during recruitment, the followers' behavior under normal leading, and finally, their behavior after leader loss. There have been sporadic reports about any two of those components having correlation, but these four variables had never been simultaneously investigated under a single question in any species of ants. This study demonstrates that *C. japonicus* has considerable variation of explorative tendency in group foraging, and there is an overarching connection that influences every stage of recruitment.

The most important limitation of this study is that the design is mostly observational, and many conclusions lack the support of controlled, manipulative experiments. Therefore, findings reported here should only be interpreted as simple correlations, not causative relationships. In order to provide solid cause-and-effect overview of this topic, it is necessary to conduct controlled experiments that can reliably reproduce different categories of the leader.

If subsequent study verifies that the associations described here are indeed causalities, it would become one of the best known example of variable foraging strategy found in a single species of ant. Variable foraging strategy attracts ecologists' attention, as it is expected to improve collective performance (Campos et al. 2016). The versatility of *C. japonicus* foraging might be the key of this species' success in East Asia. Along with the species' interesting position in

evolution of the ground-nesting trait, *C. japonicus* would provide a fertile ground for studying flexible strategies found in social insects, not limited to the scope of this work.

Table 3.1 The descriptive statistics (mean, standard deviation, minimum, 25% quartile, median, 75% quartile and maximum) measured from the variables used in the analysis.

(A) leader-present dataset, (B) leader-removed dataset.

Table 3.1A (continued)

		mean	s. d.	min	25% quart.	median	75% quart.	max
pre-recruitment variables	competition component	0	1.29	-1.33	-1.17	-0.604	1.23	1.97
	scout mobility	0.351	0.126	0.188	0.279	0.353	0.384	0.695
	number of trips	1.93	1.03	1	1	2	2	5
	time per trip	1210	663	295	866	1130	1410	2730
recruiting leader variables	mean x speed	2.979	0.846	1.629	2.334	3.03	3.72	4.26
	mean y speed	2.805	1.11	1.257	2.085	2.643	3.3	5.49
	x speed coef. of var.	0.991	0.173	0.708	0.871	0.963	1.11	1.32
	y speed coef. of var.	1.04	0.229	0.785	0.851	0.989	1.1	1.54
	y range	31.3	13.4	9.22	23.2	30.6	41.4	50
recruited follower variable	follower dispersion	14.4	8.48	4.16	8.83	11.4	18.6	47.4

Table 3.1B (continued)

		mean	s. d.	min	25% quart.	median	75% quart.	max
pre-recruitment variables	competition component	0	3.24	-1.33	-1.33	-1	3.95	6.68
	scout mobility	0.368	0.209	0.116	0.272	0.297	0.44	0.873
	number of trips	1.5	0.527	1	1	1.5	2	2
	time per trip	1450	1250	406	661	929	1860	4360
recruited follower variable	proportion of advancing followers	0.371	1.31	0	0.11	0.347	0.635	0.8

Table 3.2 Follower dispersion is probably a negative predictor of food-reach success.

Fixed-effect estimates obtained from a generalized linear mixed model, 168 observations in 14 groups.

food-reach success ~ follower dispersion + (1|experiment ID)				family = binomial	
	estimate	SE	z	p	
intercept	3.2	0.527	6.07	1.27E-09	***
follower dispersion	-0.125	0.0288	-4.33	0.000015	***

Table 3.3 Mean follower dispersion of a group is probably a positive predictor of collective search efficiency.

Estimates obtained from a weighted linear model, 14 observations.

search efficiency ~ mean follower dispersion + number of followers				weights=number of followers	
	estimate	SE	t	p	
intercept	14.7	6.7	2.2	0.0503	
mean follower dispersion	1.31	0.333	3.94	0.00233	**
number of followers	-0.569	0.115	-4.94	0.00044	***

Table 3.4 Pre-recruitment scout mobility is probably a positive predictor of follower dispersion during recruitment.

Fixed-effect estimates obtained from averaging over linear mixed models, 168 observations in 14 groups.

follower dispersion ~ number of trips + scout mobility + competition component + time per trip + (1|experiement ID)

	estimate	SE	adjusted SE	z	p	
intercept	14.3	1.04	1.05	13.6	<2E-16	***
competition component	1.37	1.07	1.08	1.28	0.201	
number of trips	1.54	1.08	1.08	1.42	0.155	
scout mobility	3.83	0.958	0.964	3.97	0.000072	**
time per trip	0.133	0.434	0.436	0.304	0.761	

All predictor variables were scaled and centered.

Table 3.5 The PCA rotation matrix extracted from the recruiting behavior of the leader. Descriptive aliases were given for convenience.

	"slow"	"varying speed"	"going sideways"	"undulating"	"stroking"
	PC1	PC2	PC3	PC4	PC5
meanXstep	-0.605	0.112	-0.330	0.564	0.440
meanYstep	-0.647	0.052	-0.270	-0.411	-0.580
cvXspeed	0.005	0.706	-0.062	-0.532	0.463
cvYspeed	0.109	0.695	0.198	0.476	-0.489
yRange	-0.450	-0.049	0.880	-0.059	0.129

Table 3.6 The explained variance of each principal components extracted from the recruiting behavior of the leader.

	PC1	PC2	PC3	PC4	PC5
standard deviation	1.428	1.316	0.866	0.670	0.172
proportion of variance	0.408	0.346	0.150	0.090	0.006
cumulative proportion	0.408	0.754	0.904	0.994	1.000

Table 3.7 The "stroking component," PC5 of the recruiting leader's behavior, is probably a negative predictor of follower dispersion of the group.

Fixed-effect estimates obtained from averaging over linear mixed models, 168 observations in 14 groups.

follower dispersion ~ PC1 + PC2 + PC3 + PC4 + PC5 + (1|experiment ID)						
	Estimate	SE	adjusted SE	z	p	
(Intercept)	14.8	0.846	0.852	17.4	2E-16	***
PC1 "slow"	0.0991	0.374	0.376	0.263	0.792	
PC2 "varying speed"	0.105	0.349	0.351	0.3	0.764	
PC3 "going sideways"	0.979	1.12	1.12	0.874	0.382	
PC4 "undulating"	0.0254	0.652	0.657	0.039	0.969	
PC5 "stroking"	-26.4	5.02	5.06	5.22	0.0000002	***

Table 3.8 The leader presence is probably a positive predictor of food-reach success of the followers.

Fixed-effect estimates obtained from a generalized linear mixed model, 301 observations in 24 groups.

food-reach success ~ leader presence + (1|experiment ID)				family = binomial	
	estimate	SE	z	p	
intercept	-1.69	0.557	-3.03	0.00247	**
leader presence	2.78	0.738	3.78	0.00016	***

Table 3.9 A model was fitted to predict the "stroking component" of a recruiting leader based on variables obtained prior to recruitment.

There was little evidence of any of the variables being a strong predictor. Estimates obtained from a linear model, 14 observations.

stroking component ~ number of trips + scout mobility + competition component + time per trip					
	estimate	SE	adjusted SE	z	p
intercept	0.52	0.76	0.849	0.613	0.54
number of trips	-0.789	0.886	0.946	0.834	0.404
scout mobility	-1.76	1.2	1.32	1.34	0.181
competition component	0.0276	0.231	0.258	0.107	0.915
time per trip	-0.0142	0.246	0.277	0.051	0.959

All predictor variables were scaled and centered.

Table 3.10. The model-predicted leader category is probably a predictor of a follower's advancing behavior after losing the leader.

Fixed-effect estimates obtained from a generalized linear mixed model, 116 observations in 10 groups.

advancing after leader loss ~ predicted leader category + (1|experiment ID)				family = binomial	
	estimate	SE	z	p	
intercept	-2.01	0.695	-2.9	0.00379	**
predicted leader category (continuous)	3.02	1.16	2.6	0.0094	**

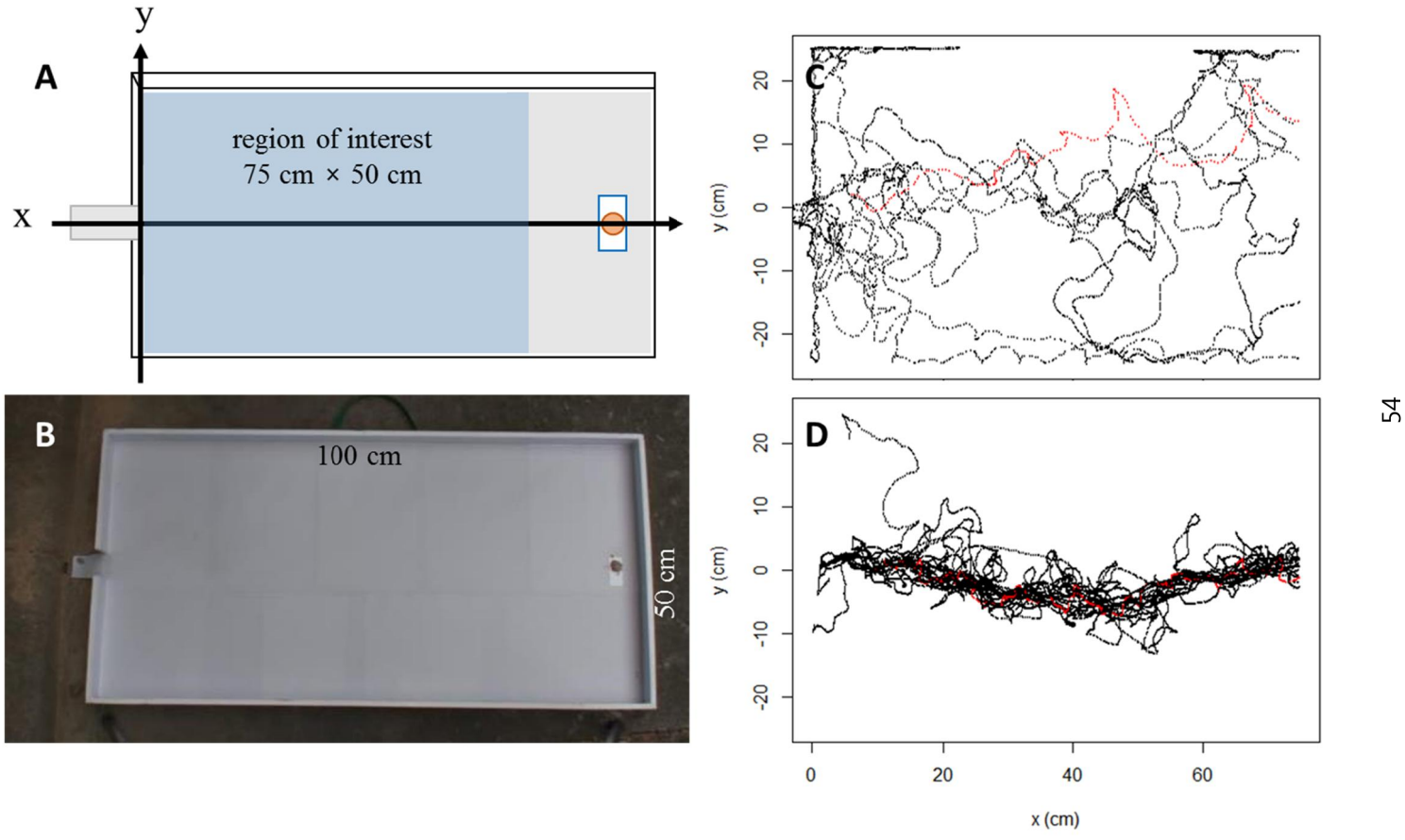
A
y
x
region of interest
75 cm × 50 cm
B
100 cm
50 cm
C
D
y (cm)
x (cm)
20
10
0
-10
-20
0
20
40
60

Figure 3.1 The experiment setup and the variation of follower dispersion.

(A) An x-y coordinate system was defined to describe the movements. A region of interest (ROI) was set to exclude the direct effect of the bait. (B) The test arena was installed in the field, in front of a wild nest of *C. japonicus*. (C, D) Example trajectory maps of highly dispersed (C) and coherent (D) foraging groups. Red dots, footprints of recruiting leaders; black dots, footprints of recruited followers.

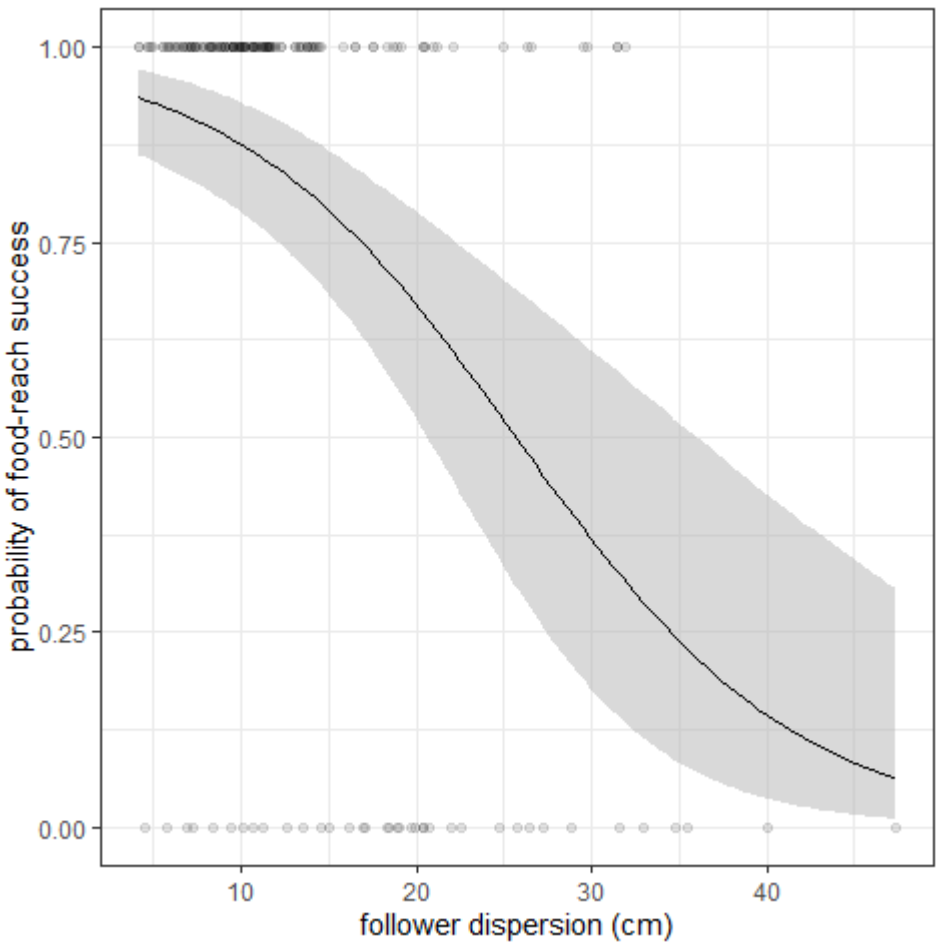

Figure 3.2 Follower dispersion is probably a negative predictor of food-reach success.

Line, model fit (**Table 3.3**); band, 95% confidence interval; dots, individual followers.

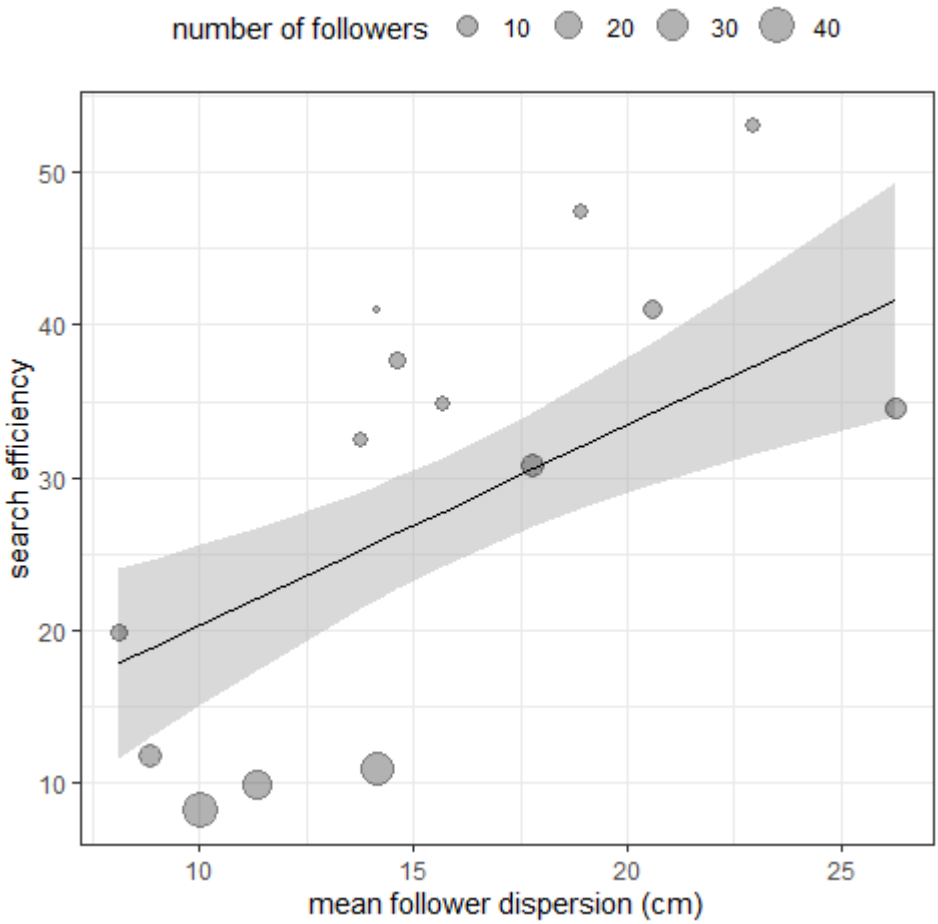

Figure 3.3 Mean follower dispersion of a group is probably a positive predictor of collective search efficiency.

Line, model fit (**Table 3.4**); band, 95% confidence interval; bubbles with different size, foraging groups visualizing the number of followers.

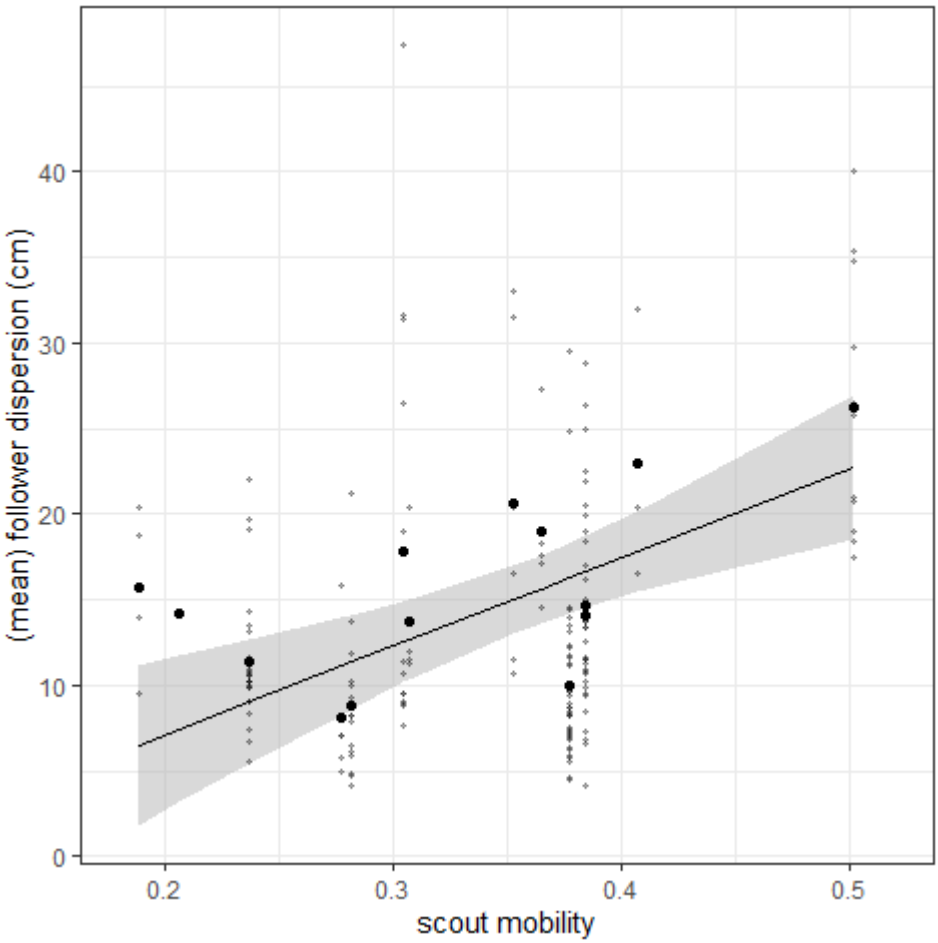

Figure 3.4 Pre-recruitment scout mobility is probably a positive predictor of follower dispersion during the recruitment.

Line, model fit (**Table 3.2**); band, 95% confidence interval; small grey dots, individual followers; large black dots, group means.

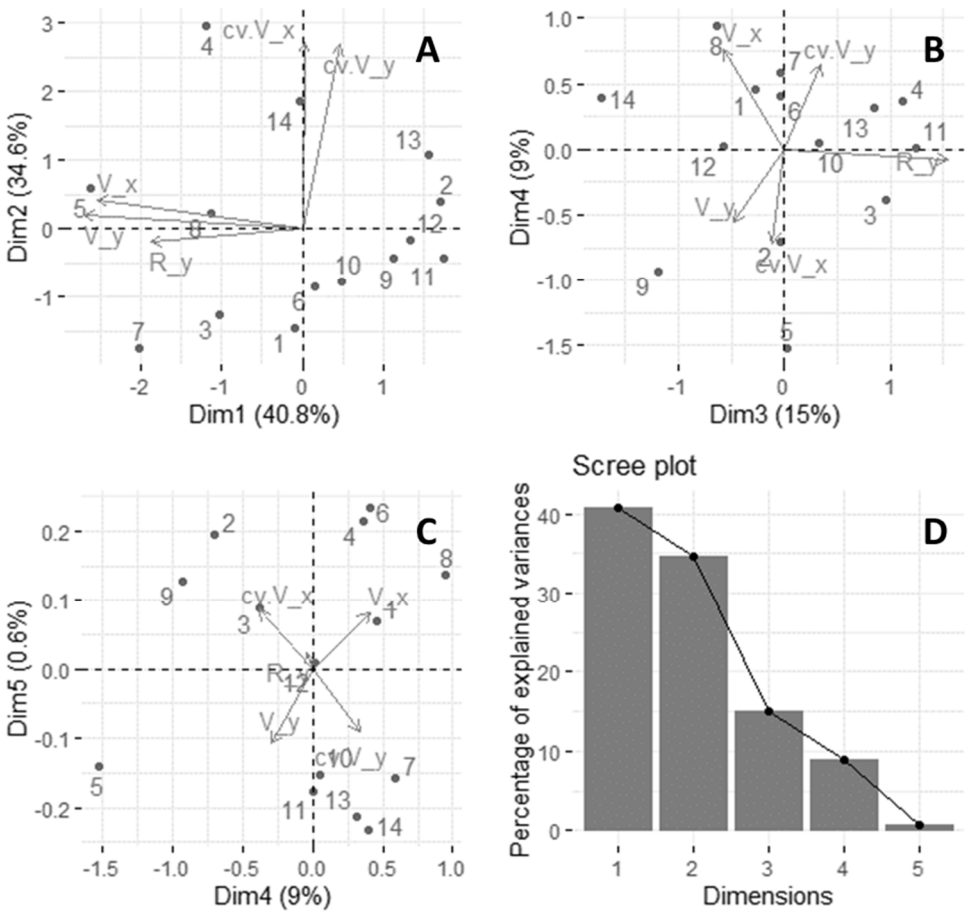

Figure 3.5 Principal components extracted from the leaders' recruiting behavior.

(A-C) Biplots visualizing the rotation matrix of each component. Dots with numbers, individual leaders with experiment ID. (D) A scree plot showing variances explained by each component.

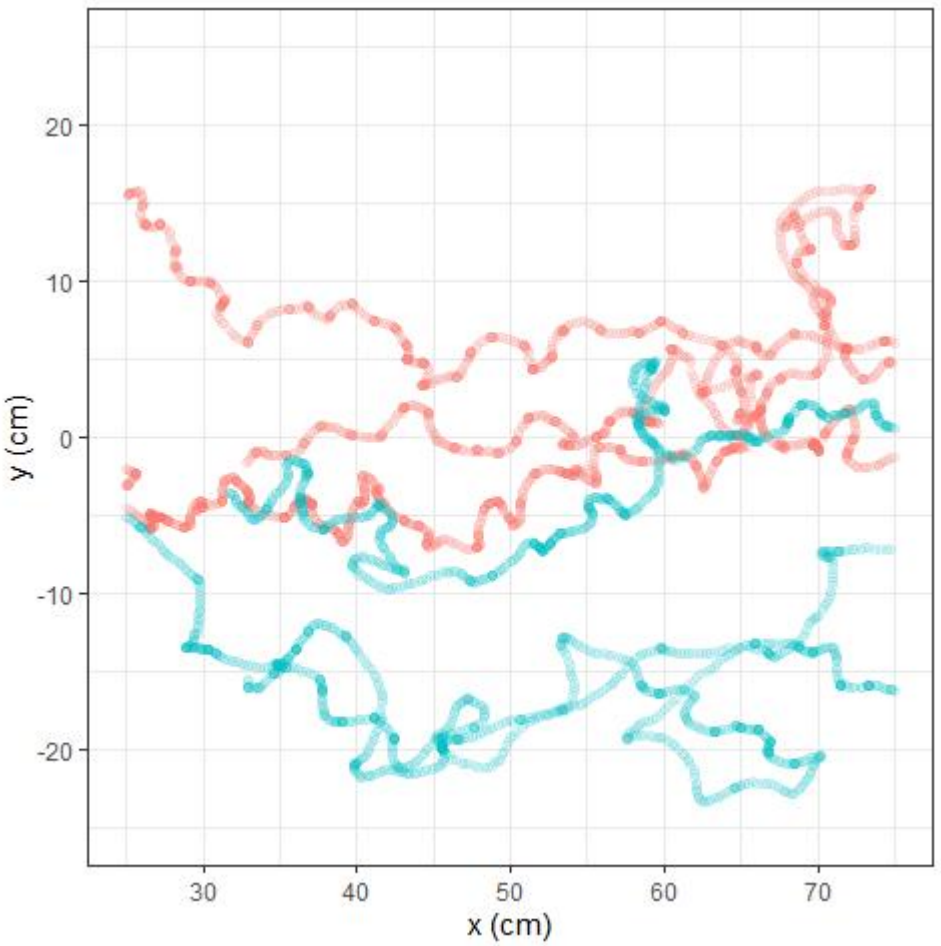

Figure 3.6 Example leader trajectories with high and low values of principal component 5 (PC5; "stroking" component).

Footprints from three leaders with the highest PC5 (red dots) and three others with the lowest PC5 (blue dots) are presented. Footprints are depicted translucently, and therefore thick-colored locations show the positions where the leader slowed down.

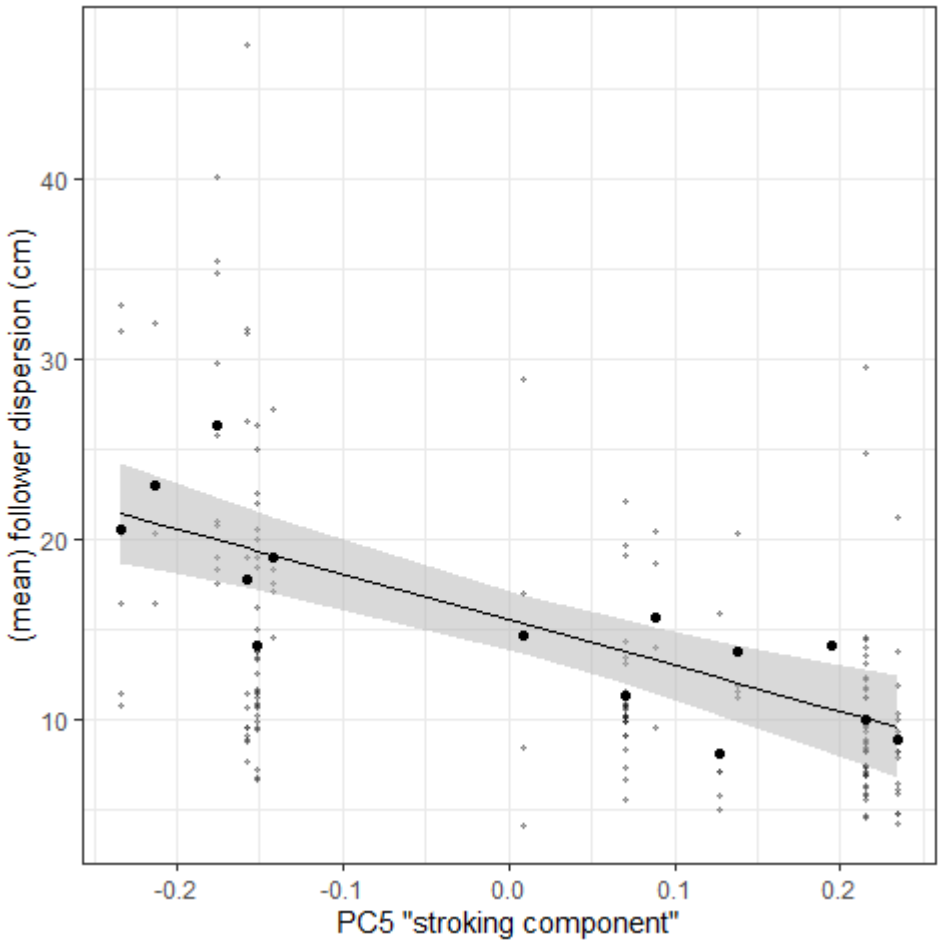

Figure 3.7 Leader's recruiting behavior component "stroking (PC5)" is probably a negative predictor of follower dispersion.

Line, model fit (**Table 3.7**); band, 95% confidence interval; small grey dots, individual followers; large black dots, group means. Note that the leader's stroking component (x-axis variable) is bimodally distributed.

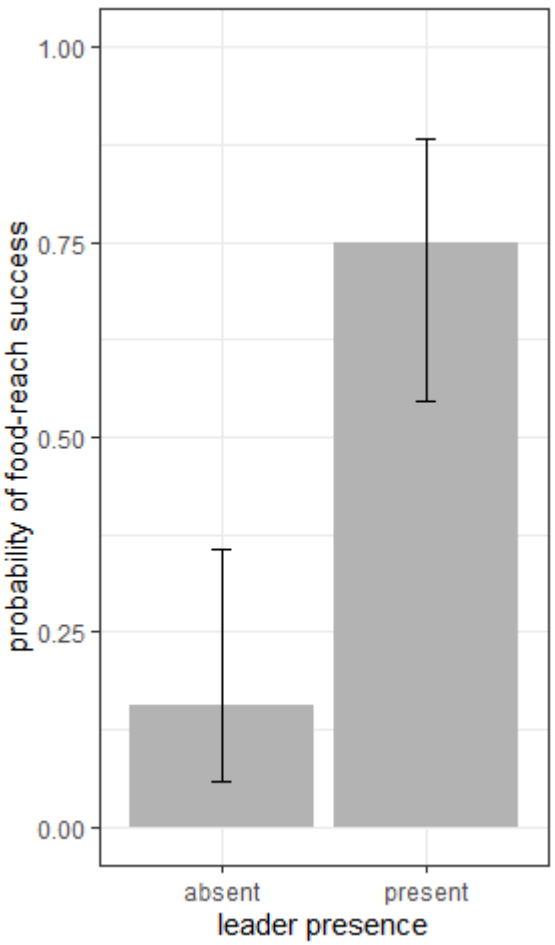

Figure 3.8 Leader presence is probably a positive predictor of food-reach success of the followers.

Solid bars, model fit (**Table 3.9**); error bars, 95% confidence intervals.

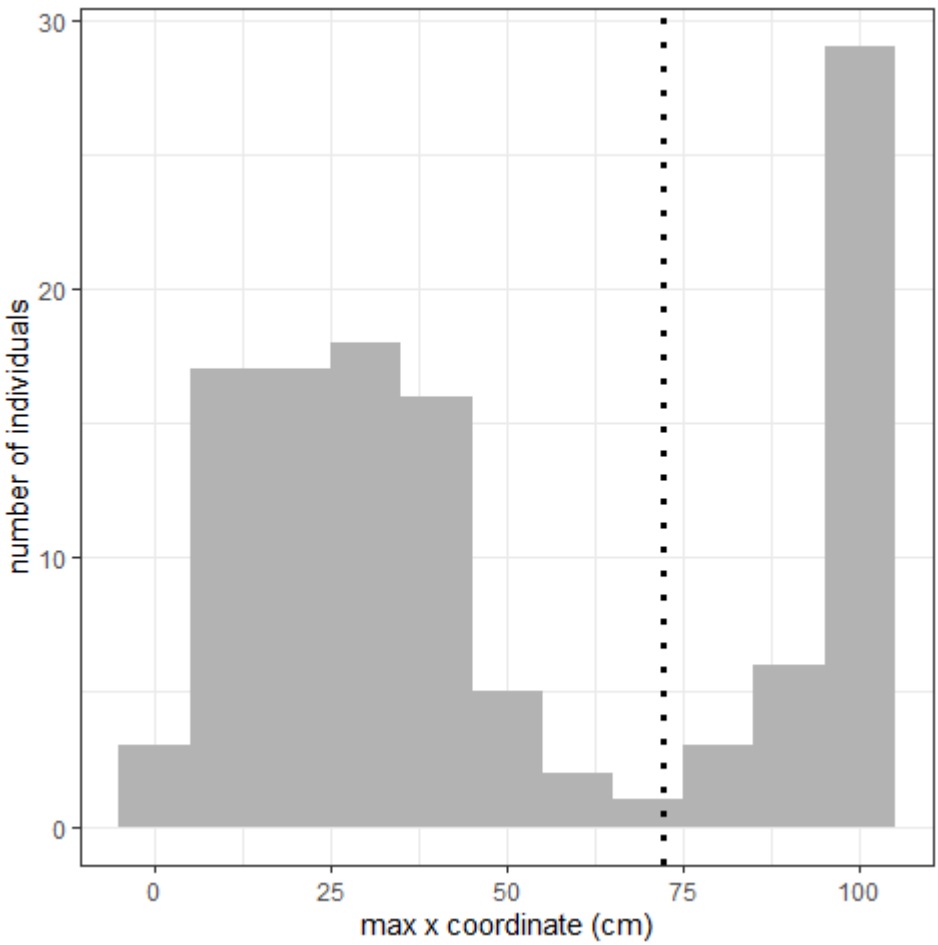

Figure 3.9 Abandoned followers probably have binary responses after leader loss.

Histogram, maximum x-coordinates reached by followers; dotted line, separation criterion between the two clusters obtained from Ward-D hierarchical clustering.

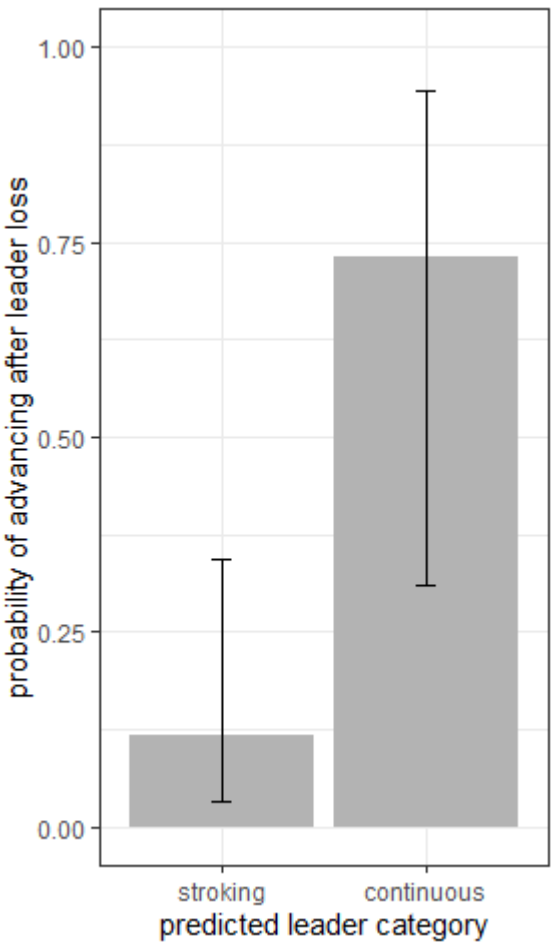

Figure 3.10 Model-predicted leader category (Table 3.8) is probably a predictor of the remaining followers' advancing behaviors after leader loss.

Solid bars, model fit (**Table 3.10**); error bars, 95% confidence intervals.

CHAPTER 4. SWITCHABLE APOSEMATISM: A BEHAVIORALLY CONTROLLED WARNING SIGNAL

In the previous two chapters, I explored the flexibility found in foraging strategies of the ants, in both ultimate and proximate perspectives. After concluding my first topic, I now move on to my second theme of the study: the behaviorally controlled ('switchable') aposematism.

Ideally, it would have been great if I could cover both ultimate and proximate mechanisms of switchable aposematism, as I have done for the previous topic. However, this thesis only covers the ultimate evolutionary mechanism of aposematic switchability; due to practical limits, my study concludes without enquiring proximate mechanisms. It remains as an important future question that should be addressed after this work.

In this chapter, I attempted to provide a single, unified theoretical context to a number of proposed evolutionary mechanisms that are potentially relevant to the origin of the switchable aposematism. This quest culminated to development of *ApoSim*, an agent-based evolutionary simulation model with extraordinarily many parameter dimensions. This chapter is merely an example case of its usage, and the evolution of switchable aposematism still leaves a lot of unanswered questions even after the contributions made in this chapter.

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journal could be reversed in order. In case of acceptance and publication in *PeerJ*, the article will be copyrighted under Creative Commons CC-BY 4.0 License according to the journal policy.

4. 1 Introduction

The switchable aposematism.

Some prey animals possess defensive measures that make them unprofitable to predators. Such defended prey often signals their unpalatability via bright colors, alarming sounds, or other conspicuous components. These signals can affect predator's decision to accept the prey because the predator learns association between the signal and the prey unprofitability. This form of anti-predatory strategy is called aposematism. Evolutionary biologists have explored various dimensions of aposematism, but its behavioral aspect had not received adequate attention until recent years. While some aposematic signals such as permanent coloration are fixed and operate continuously, other signals can be behaviorally controlled, by sound generation (Dowdy & Conner 2016), wing movement (Kang et al. 2016a), bioluminescence (De Cock & Matthysen 1999), physiological color change (Umbers et al. 2014), or postural change (Lariviere & Messier 1996). I will call this form of aposematism as the "switchable aposematism."

Historically, the switchable aposematism has been described with various adjectives such as "facultative," (Sivinski 1981; Grober 1988) "post-attack," (Umbers & Mappes 2015; Kang et al. 2016a) "early-acting," (Broom et al. 2010) "deimatic," (Umbers et al. 2015; Umbers & Mappes 2015) or "switchable," (Umbers et al. 2017) implying various functional or temporal characteristics found

in each study species. This had led to disputes regarding the use of "deimatic" as either descriptor of function or that of a form (Umbers et al. 2015; Umbers & Mappes 2015; Skelhorn et al. 2016; Umbers et al. 2017). In this article, I chose to use a term that indisputably concerns the form of the display behavior: 'switchable.'

The renewed interest in the switchable aposematism.

In contrast to the well-developed knowledge on switchable displays in undefended (profitable) prey in the context of "startling" or "deimatic" function (sudden display surprising the predator away: Edmunds (1972); Schlenoff (1985); Grandcolas & Desutter-Grandcolas (1998); Vallin et al. (2005); Bura et al. (2011)), the switchable aposematic signals of defended prey have been largely ignored. Notable exceptions are early studies on bioluminescence in defended prey (Sivinski 1981; Grober 1988) and short descriptions of switchable conspicuous signals in some defended animals (Robinson 1969).

However, recently researchers started experimentally exploring the switchable visual displays in some of chemically defended prey (Kang et al. 2011; Umbers & Mappes 2015; Kang et al. 2016a) rising new questions and "rejuvenating" old ones in this field (Umbers et al. 2017).

Costs and benefits of the switchable aposematism.

Physiological and/or anatomical mechanisms that allow signal switching might impose additional costs to the prey animals. Some of these costs might involve the development and maintenance of such mechanism ("fixed" cost), while others might be paid every time the switching behavior occurs ("per-use" cost, terminologies adopted from Broom et al. (2010)).

On the other hand, the switchable signal can be beneficial in several ways. First, regardless of any aposematic function, a sudden switch may simply surprise the predator away (the "startling" effect (Ruxton et al. 2018)). The sudden switching might also accelerate the predator nervous systems' learning about the aposematic prey (Kang et al. 2016a). Finally, it might cut down unnecessary signal exposure because an animal can present the expensive signal only when needed (Grober 1988; De Cock & Matthysen 1999; Umbers et al. 2017).

Among these benefits, the cost-reducing aspect is probably the most cumbersome to handle in empirical studies because the evolutionary cost of a signal is difficult to quantify or manipulate (Srygley 2004; Bohlin et al. 2008; Crothers et al. 2011; Lindstedt et al. 2016). Therefore, I chose to use a modeling approach to focus on the variety of costs of the switchable signals.

Different types of costs related to the switchable aposematism.

In order to avoid confusion between the different types of costs involved in switchable aposematism, I will use consistent specific expressions from now on. The energetic or material cost paid per every switching will now be called the 'switching cost.' The developmental and/or maintenance costs associated to the switching mechanism itself, which is always paid regardless how often the switching-signal is actually fired, will now be collectively called the 'switchability maintenance cost.' These two types of costs are expected to hinder the evolution of switchability. The signal-induced exposure to the predators will now be called the 'detectability cost.' All the other costs inherent to the signal itself, e. g. energy consumption for producing sound, will now be called the 'signal penalty.' These

two types of costs are expected to hurdle the evolution of aposematism itself, but switchability may help relieve them. A generic term that encompasses and summarizes all of these negative effects will be the 'general cost(s) of signaling.' This general cost is expected to be optimized against the general benefit of the signaling. Some of these terminologies will begin to appear in bold texts, indicating that the corresponding concept is directly modeled by a simulation variable.

Introducing *ApoSim* and its usage in this article.

I developed *ApoSim*, an individual-based simulation model to study this subject. In this model, computer-simulated predators and prey interact with each other, and their performances are under selection pressure. *ApoSim* is capable of exploring a very wide parameter space, but for this paper I assumed a special situation; the learning facilitation and startling effects of switchable signals are negligible. Under this assumption, I could explore the cost-reducing aspect of the switchable aposematism in a clean, conservative scope. Users of the model software can easily conduct future studies in order to address questions regarding the startle effect or the learning facilitation (see **Appendix**).

Previous knowledge pertaining to the general costs of switchable aposematism.

Throughout the development of *ApoSim*, I had to consider a variety of relevant phenomena that have been suggested or reported. The sequential nature of post-attack switching that is dependent on the success of the pre-attack defensive measures (Broom et al. 2010; Ruxton et al. 2018), the baseline detectability of non-

signaling prey (De Cock & Matthysen 1999; Broom et al. 2010), the fixed and per-use costs of switchability (Higginson & Ruxton 2009; Broom et al. 2010), and sensory and cognitive ability to detect predator's presence (Broom et al. 2010) have all been under investigation. The interactions between these mechanisms and the general costs of the switchable aposematic signals is not fully understood.

The general cost of switchable signaling is not a single, readily measured value, but a result of highly complex and relatively unexplored interactions. Energy consumption (Srygley 2004; Lindstedt et al. 2011), predator learning and behavior (Guilford 1994; Gamberale-Stille & Guilford 2004; Halpin & Rowe 2017), the portion of the predators not deterred by the signal (Williams et al. 2003; Mappes et al. 2014), the level of crypsis achievable when not signaling (Bohlin et al. 2008; Willink et al. 2013; Umbers & Mappes 2015), and many other aspects could all affect the general cost of signaling. Furthermore, some of these conditions can change every time the signal is switched on. Due to this complexity, the study on the switchable aposematism is still in its beginning stage. Therefore, I carefully designed the simulation so that it can embrace most of the above ideas in one framework.

Scope of this study.

Based on my simulation, I suggest new hypotheses regarding evolution of the switchable aposematism and test existing hypotheses in a context different from which they were originally formulated in. First, I explore if variation in the selective penalty of the aposematic signal is sufficient to explain the observed diversity of switchable aposematism. Second, I determine how evolutionary success of different types of switchable aposematism is affected by increasing costs

of switching. Third, I evaluate the existing hypotheses suggesting that moderate-range predator learning capabilities create the most favorable conditions for the evolution of aposematism. Fourth, I explore some previously proposed hypotheses about the effects of mixing of naïve and experienced predators on the evolution of various aposematic strategies. Fifth, I determine if and how the basal detectability of the prey affects evolution of aposematism. Finally, I explore several possible evolutionary pathways that can lead to switchable aposematism from non-switching and/or non-aposematic initial population.

Additionally, I provide my model and the source code so that the users can further explore the evolution of complete diversity of aposematic strategies. The model can simulate the interactions between these evolutionary components in a multidimensional evolutionary space. In addition, I provide a description of how to use this software to explore variables other than those used in this article.

4. 2 Methods

Core ideas and assumptions.

The prey behavioral strategy.

I modeled the prey **behavioral strategy** as responses to three conditions: "resting," being "approached," and being "attacked." Specifically, a strategy was defined by three values of **signal intensity** (ranging from 0 to 1) each associated with one of the three conditions above. For example, a strategy could be written as the following: *[(resting, 0.1), (approached, 0.1), (attacked, 0.7)]*. Each number represents the **signal intensity** given in each condition. This strategy is a variant of

post-attack switchable signaling, because the sudden increase in **signal intensity** (from 0.1 to 0.7) occurs once the predator attacks. Meanwhile, a permanently aposematic prey should have the same **signal intensity** for all three conditions. In the case of a pre-attack switcher, it should remain largely silent while resting, but switch to higher intensity when "approached." It should be noted that the **signal intensity** is a one-dimensional value; I assumed that all signal varieties operate on the same common axis, and that the predators automatically generalize their knowledge about one signaling prey to all the others. Therefore, all prey signals are 'mimetic' to each other.

While the **signal intensity** could technically take any value from 0 to 1, I further simplified this variable for the sake of easier visualization. I assumed that the prey can only have three discrete levels of signals: "none" (N), "low" (L), and "high" (H) (the exact value of each level can be adjusted by the user). With these discrete levels, one could describe a **behavioral strategy** without using the numerical values for the **signal intensity**. For example, the pre-attack switchable aposematism could be written as *[(resting, N), (approached, H), (attacked, H)]*.

I devised a three-letter annotation system as a shorthand for each **behavioral strategy**. The sequence of the three conditions was easy to remember as they are in the logical order of increasing threat level: resting, approached, and attacked. Therefore, the previous expression could be shortened to [NHH]. Likewise, one could easily understand that LLL is a permanent-display strategy with low signal intensity (L). Similarly, NLH is a strategy of an animal that does not signal at rest (N), responds to an approaching predator by switching to a weak pre-attack signal (L), and responds to a predator attack with a stronger signal (H). With this concise style, I could easily express complex ideas such as "competitive

advantage of NLL over NLH" or "the change in LLL-NNH balance in the presence of NLLs" without repeating the lengthy explanations every time.

In summary, three signal intensity levels (N, L and H) were allowed in each of the three states (resting, approached and attacked), resulting in $3^3 = 27$ possible **behavioral strategies**. The user could configure whether a specific strategy can exist or not in the model.

The predator attack event.

Given the basic structure of the prey **behavioral strategies**, it was clear that the predator should have at least two attack opportunities. The post-attack switchable aposematic signal should be fired after an attack event, and it should affect the prey survival by influencing the chance of the subsequent attack(s).

Therefore, I assumed that there are **initial attack** and **final attack** events. The **initial attack** event occurred after the prey's pre-attack behavior and before the post-attack behavior; the **final attack** event occurred after the post-attack behavior. These two attack events act as junctions that naturally divide the whole interaction into three **time steps:** pre-initial-attack period (step 1), inter-attack period (step 2), and the post-final-attack period (step 3).

The scope of the simulated evolution.

I simulated competition for higher survival among different prey **behavioral strategies**. In contrast, properties of the predator population are directly specified by the user. Hence, rather than simulating the predator-prey coevolution, the model focuses on the evolution of prey population given a certain fixed set of predator

properties. With this approach, I could explore the direct effect of specific characteristics of the predatory guild.

Overview of the model structure.

I built the model with NetLogo 5.3.1, an agent-based modeling framework developed at Northwestern University (Wilensky 1999). The simulation code and graphical interface are published with the article. Note that the graphical interface was used only for testing, prototyping and pedagogical use; the exploration of the parameter space was programmatically executed by *BehaviorSpace*, a parameter-search tool bundled with NetLogo 5.3.1.

My model, *ApoSim*, is capable of modeling startle and learning facilitation effects of switchable signals as well as a number of other ecological variables. However, here I describe only the mechanics relevant to my study questions. For the full description of the model variables, please refer to the **Appendix** and the source code.

The modeled world consisted of a single species of 180 defended prey animals and a single species of 45 predators (**Figure 4.1**). Each individual prey occupied one grid square of the 2-dimensional world (14 × 14 grids). The prey distribution was globally random, and the model did not aim to simulate the kin-selection effect of gregariousness. Initially the prey population was a uniform mix of all possible **behavioral strategies**.

The basic time unit of the simulation was the **interaction frame** (**Table 4.1**). 50 **interaction frames** formed one prey generation, after which the current prey individuals reproduced and died out. Each interaction frame included three **time steps**. In each **time step**, each prey and predator individuals followed specific

set of steps coded in program's procedures. The names of the procedures, as they appear in the source code, are given in **Table 1** and are detailed in the subsequent sections.

During each **interaction frame**, a predator could encounter and interact with an individual prey throughout the three **time steps**. During the interaction, the predator can detect the prey's **repulsive taste**, which reduces the predator **motivation**. The exposure to **repulsive taste** could lead to accumulation of the predator's **aversive memory** value from 0 to unlimited level. After enough interactions, the heightened level of **aversive memory** could cause the predator to refuse to attack this prey species. Also, the interaction could lower the **survival chance** of the prey, which began as 1 and then subsequently decreased by multiplying by decimal modifiers. Actions of the predator and the prey determined the levels of the modifiers. Depending on how the interaction unfolded, predator might fail to detect the prey or decide to abandon it. In such a case, the interaction was prematurely terminated, and the predator would idle for the rest of the **interaction frame**.

After 50 interaction frames (one prey generation) all prey entered the reproduction stage. First, the prey individuals died out according to the final values of **survival chance**. All the survivors had equal chance of reproduction. Reproduction was simulated by repeating the procedure of randomly choosing one of the survivors and generating one offspring of the same behavioral strategy until the carrying capacity (180 individuals) of the next generation was reached. After the entire reproduction stage was over, the survivors of the current generation died immediately (non-overlapping generations). There was no simulation of offspring

growth stages. There was no sexual reproduction, and each offspring exhibited the parental phenotype identically.

On the other hand, the predators did not have any fixed lifespan, and a preset number of fresh naïve predators randomly replaced the old individuals according to the user-defined **predator turnover** rate at the end of each prey generation. I assumed that predators acquire the avoidance entirely through associative learning, not instinct. The predator **learning speed** was adjustable by the user. The predators were genetically homogeneous and not subject to natural selection.

The detailed simulation structure.

Interaction frame: time step 1.

"Predators-move" procedure. - At the beginning of each interaction frame, every predator moved to a random grid square which contained a prey. In my setup, the number of the predators was smaller than that of prey; this procedure left the majority of the prey without predator encounter.

"Prey-react" procedure. - Each prey detected the predator presence or absence in the grid square it was occupying, and it then switched or maintained its aposematic signal. After detecting the predator absence or presence, the prey then stayed still or switched its **signal intensity** depending on its **behavioral strategy**. More specifically, each prey would enter or continue "resting" state if no predator was in its grid square, and if one was present, then the prey would assume the

"approached" state instead. As detailed in the first section of **Methods**, the prey **signal intensity** was one of the three levels: none (N), low (L), or high (H). Each level corresponded to a number ranging from 0 (minimum possible signal) to 1 (maximum possible signal), and the exact values could be set by the user.

In this study, I assumed that the prey had perfect sensory and cognitive ability to accurately determine the predator presence. I do recognize that ambush predators are major exceptions to this assumption, and therefore I do not intend to explain their ecology with my model.

"Prey-pay-cost" procedure. - Regardless whether it was encountering a predator or not, each prey had to pay the energetic and ecological price of its current behavior and appearance.

First, if the prey had switched the signal to a different level, such behavior might require extra energy and reduce its chance of survival. The user-adjusted variable **switching cost** (range 0 to 1) represented this effect, and the prey **survival chance** (range 0 to 1; initially at 1) decreased accordingly (**Eq. 1**). Note that I are using Roman numerals to mark the intermediate values used in sequential calculations (an example is "survival chance I" below). Also, I decided to use full-word names of the variables instead of single letter symbols because I think this will facilitate better and easier understanding of the whole model by the intended readership – biologists who study aposematism.

Eq. 1 survival chance I = current survival chance * (1 – switching cost)

Second, an animal with a switching mechanism needs to maintain it energetically and cope with the developmental complexity regardless whether the actual switching behavior has fired or not. In my model, a user-adjusted variable **switchability maintenance cost** (range 0 to 1) simulated this effect (**Eq. 2**).

Eq. 2 survival chance II = survival chance I * (1 – switchability maintenance cost)

Finally, the signal itself, be it switchable or permanent, might impose a variety of burden of prey survival that are not explicitly modeled in my software. For instance, high **signal intensity** could attract parasites that are not deterred by the unpalatability. If the prey's mode of signaling involved sound generation or odor production, there would be extra energy consumption. If the prey uses a physical device that can be shown, moved, or inflated, the prey animal's mobility would suffer in addition to energy costs. A myriad of such effects were summarized in my model as **signal penalty**, a user-adjustable value from 0 to 1. This was weighted by the **signal intensity** (**Eq. 3**).

Eq. 3 new survival chance = survival chance II * (1 – signal penalty * signal intensity)

In principle, there would be a production and maintenance cost for all signals regardless switchability; some developmental burdens such as pigment production could be shared by permanent and switchable signals. I decided to exclude this

variable from the model, as its effect is largely trivial and predictable – the costlier the signal maintenance, the lower the prevalence of signaling phenotypes.

"Predators-search" procedure. - Each predator investigated the grid square and detected the prey presence with a certain probability. This **discovery chance** (**Eq. 4**) was a function of **basal detectability** and the **signal intensity** of the prey. The **basal detectability** (range 0 to 1; user-adjusted variable) was a simplified representation of prey body shape, the habitat properties, the predator cognitive attributes, and anything that can affect the minimum level of **discovery chance**. Hence, the **discovery chance** value was larger for higher **basal detectability**. It was additionally increased if the **signal intensity** value was larger than 0. The **signal intensity** effect was weighted by the maximally possible increase of the detectability (1 – **basal detectability**), so the final value was limited between 0 and 1. Unlike many other similar equations in the model, the **basal detectability** and **signal intensity** has additive relationship, not multiplicative. The rationale behind this design is to prevent one side being zero from negating the other; each of them should be able to invoke predator discovery even if the other one is very small.

Eq. 4 discovery chance = basal detectability + signal intensity * (1 - basal detectability)

"Predators-observe" procedure. - If a predator had detected a prey, then it observed the appearance of the target prey. The **conditional stimulus** (range 0 to 1) was a value that represented the intensity of all information that can mediate the predator's associative learning regarding the prey. A high **conditional stimulus**

value meant that the prey had characteristics that allow easier memory buildup and recall.

The information available to predator could have been come from either the prey's signal or its signal-independent inherent traits. The **conditional stimulus** was a function of prey **signal intensity** and **basal cognitive cue** (**Eq. 5**). Note that the formula is in the same format as the **Eq. 4**; the **basal cognitive cue** sets the baseline, and the **signal intensity** determines the remaining portion so that the final value cannot exceed 1.

Eq. 5 conditional stimulus = basal cognitive cue + signal intensity * (1 - basal cognitive cue)

The prey body shape, the predator cognitive attributes, environmental conditions, and many other factors could govern the minimum level of associative learning and memory retrieval. The **basal cognitive cue** (range 0 to 1; user-adjusted variable) represented them. Note that the **basal detectability** and the **basal cognitive cue** were two different variables despite looking similar. The basal detectability affected the chance of discovery (**Eq. 4**), while the **basal cognitive cue** was for the learning/recalling stimulus (**Eq. 5**). For example, a flying insect might be easily detectable (high **basal detectability**) but it could be difficult to learn or recall from the appearance, because it looks too similar to many other profitable insects (low **basal cognitive cue**).

"Predators-analyze" procedure. – From the information observed from the prey, the predator attempted to determine its attractiveness based on memory as well as taste

(if available). After perceiving the **conditional stimulus**, each predator assessed **prey attractiveness**. The **prey attractiveness** (range 0 to 1) was a function of **learned repulsiveness** and **instinctive repulsiveness**, both with a range from 0 to 1 (**Eq. 6**). For a prey to be highly attractive, both learned and instinctive repulsiveness should be close to 0.

Eq. 6 prey attractiveness = (1 - learned repulsiveness) * (1 - instinctive repulsiveness)

The predator's impression toward the prey was determined by the level of memory, the strength of cues that recall the memory, and the currently felt taste (if available). The **learned repulsiveness** was determined by the level of the **conditional stimulus** (**Eq. 5**) and the **aversive memory**. The learned repulsiveness was forced to be within the range of [0, 1], as seen in **Eq. 7**. The **instinctive repulsiveness** was solely determined by the **unconditional stimulus** (range 0 to 1) the predator was currently feeling (**Eq. 8**). Please note that the **unconditional stimulus** is merely a continuously updated sensory input, not a stored memory. Also, the model software can be configured to include the 'startle' effect in the **instinctive repulsiveness** calculation; see **Appendix**.

Eq. 7 learned repulsiveness = minimum $\begin{Bmatrix} 1 \\ aversive\ memory * conditional\ stimulus \end{Bmatrix}$

Eq. 8 instinctive repulsiveness = unconditional stimulus

The predator's past memory and the cues that recall it were both necessary for a learned avoidance to take effect. Unlike all the other values that range from 0 to 1, **aversive memory** could be any non-negative number and had no upper bound. Therefore, sufficiently high **aversive memory** could bring the **learned repulsiveness** up to 1 even if the **conditional stimulus** (**Eq. 5**) was very small. On the other hand, a completely naïve predator with no **aversive memory**, will have no **learned repulsiveness** regardless of the **conditional stimulus**. The mechanics of the **aversive memory** buildup will be detailed in the subsequent sections (**Eq. 11a, b**).

The taste information is only accessible after the predator made at least one attack event. The **unconditional stimulus** represented all modes of unprofitability that can be sensed by the predator during attack events. In the current **time step 1**, the predator had not yet tasted the prey, so the received **unconditional stimulus** was 0. In the later time steps, there will be taste information available, and the mechanics will be detailed in the subsequent sections.

"Predators-decide" procedure. – From the predator's impression of the target prey, it probabilistically decided to attack it or not. The **motivation** (range 0 to 1) began from 1 whenever the predator encountered a new prey. After assessing the **prey attractiveness** (**Eq. 6**), each predator modified its **motivation** according to it. In order to maintain high **motivation**, the **prey attractiveness** should remain close to 1 (**Eq. 9**).

Eq. 9 new motivation = current motivation * prey attractiveness

This value ("new motivation"), with range 0 to 1, was the probability of deciding to attack the focal prey in the next **time step**.

Interaction frame: time step 2.

"Predators-initial-act" procedure. - Each predator conducted an action (attack or abandonment) following the "decision" made in the previous step. The probability of attack was the value of "new motivation" (Eq. 9.) at the end of the **time step** 1. If the predator decided not to attack, then it abandoned the prey and remained idle for the rest of the **interaction frame**. If the predator decided to attack, the predator-prey pair underwent an **initial attack** event. In the event, the prey suffered a decrement in its **survival chance** (range 0 to 1). This effect was governed by the user-adjusted variable **initial attack damage** (range 0 to 1; **Eq. 10**).

Eq. 10 new survival chance = current survival chance * (1 – initial attack damage)

"Predators-feel" procedure. - After attacking a prey, each predator received taste information from handling the prey. Therefore, the predator could now update the **unconditional stimulus** value. In the current **time step 2**, the predator is performing its **initial attack**, so the information is updated from 0 to the **repulsive taste** (range 0 to 1; user-adjusted variable) of the prey. I assumed that the prey **repulsive taste** is a homogeneous characteristic across all prey, and there was no mutation or variation modeled. See **Discussion** for the implications of this assumption.

"Prey-react" procedure. - After being attacked or abandoned by a predator, each prey could now be in the "attacked" or "resting" state, and reacted to the situation. The details were identical to the "prey-react" procedure of the **time step 1**. Based on its **behavioral strategy** (as described in the first section of **Methods**), the prey changed its **signal intensity** or remained in the current appearance.

"Prey-pay-cost" procedure. - Each prey underwent a series of **survival chance** decrements, following the principles identical to the **time step 1** (**Eq. 1, 2, 3**). Especially, if the reaction involved switching of **signal intensity** to a different level, the prey paid the **switching cost** (**Eq. 1**).

"Predators-observe" procedure. - As it did in the previous **time step 1**, each predator experienced the **conditional stimulus** (**Eq. 5**) again, but this time calculated with the current value of **signal intensity** displayed in step 2 (after predator initial attack).

"Predators-learn" procedure. The predator learned the association between the prey and the negative taste, and the speed of learning was determined by how striking the prey's appearance was, how unpleasant the taste was, and how the program user set the global adjustment to all learning process. Technically speaking, each predator built up **aversive memory** (**Eq. 11a, b**) based on the newly updated **conditional stimulus** (**Eq. 5**), **unconditional stimulus** (see the "predators-feel" procedure above), and a user-adjusted coefficient called **learning speed**.

Eq. 11a new aversive memory = current aversive memory + aversive memory increment

Eq. 11b aversive memory increment = learning speed * repulsive taste * conditional stimulus

The **aversive memory** was any non-negative value with no upper bound, as explained in **Eq. 7** and the accompanying text.

For the sake of simplicity, there was no memory decay modeled, and the predator population turnover was the only source of collective memory decrement. I made this assumption because taste aversions tend to have very high retention time (Steinert et al. 1980; Elkins 1984). It is possible for the users to modify the code and include memory decay if desired.

I allowed the speed of learning to be adjusted over very wide range. Similar to the **aversive memory**, the user-adjustable **learning speed** also had no upper bound; very fast learning could induce substantial build-up of **aversive memory** even when both **unconditional stimulus** and **conditional stimulus** were fairly weak. For perspective, I used **learning speed** values from 0 to 1000 in my study. The **learning speed** was an abstraction of the predator sensory and cognitive abilities as well as the environmental difficulties that might hinder acquisition of the aversion. For example, if Batesian mimics or other harmless yet similar-looking food sources were present in the habitat, the predator aversion learning would require longer time. The user might want to change the **learning speed** setting to form hypotheses involving such effects.

"Predators-analyze" procedure. - Each predator used the updated **aversive memory**, **conditional stimulus**, **unconditional stimulus** values to determine the **prey attractiveness** (**Eq. 6**) again.

"Predators-decide" procedure. - Each predator updated its **motivation** (**Eq. 9**) based on the reassessed **prey attractiveness**. The new **motivation** value was the probability to attack the prey in the next **time step 3.**

Interaction frame: time step 3.

"Predators-final-act" procedure. It was almost identical to the "predators-initial-act" procedure in the previous time step. This time its action was either a **final attack** event or an abandonment, and the prey **survival chance** was affected by **final attack damage** (range 0 to 1), another user-adjusted variable (**Eq. 12**).

Eq. 12 new survival chance = current survival chance * (1 – final attack damage)

"Predators-feel" procedure. Each predator received the updated **unconditional stimulus** as it did in the previous **time step**. Since this is the **final attack**, the information would not be different from what it felt in the **initial attack**.

"Prey-react" procedure. - Each prey reacted to the **final attack** in the same manner it did in the previous **time step 1** and **2**, following its **behavioral strategy** (see the first section of **Methods**).

Note that, in some situations, the prey would gain little benefit from reacting to the last attack move from the predator; the predator is going to leave the site afterwards, regardless of the prey response. However, I assumed that the prey cannot know if the predator interaction is coming to an end, and that it will unconditionally display the signal as long as its **behavioral strategy** assigns a response to attack.

In other situations, strongly reacting until the end of the attack (and thus giving prolonged stimulus to help predator learning) may increase prey fitness, if the prey or its offspring was likely to encounter that identical predator individual again in the future.

"Prey-pay-cost" procedure. - Each prey suffered drops in **survival chance**, as it did in the previous **time step 1** and **2** (**Eq. 1, 2, 3**).

"Predators-observe" procedure. - Each predator gathered the **conditional stimulus** (**Eq. 5**) based on the updated **signal intensity** information, as it did in the previous **time step 1** and **2**.

"Predators-learn" procedure. - Each predator incremented the **aversive memory** (**Eq. 11a, b**) with the updated **conditional** (**Eq. 5**) and **unconditional** (see the "predators-feel" procedure) **stimuli** as well as the user-adjusted **learning speed**.

Reproduction stage.

"Prey-selectively-die" procedure. - Each prey survived or died according to the final value of its **survival chance**.

"Prey-lay" procedure. - One of the surviving prey individuals was randomly chosen to generate a clone that would live in the next generation. The process was repeated until the carrying capacity (180 individuals in my case) of the next generation was all filled.

"All-prey-die" procedure. - Every prey of the current generation died out, only leaving the next-generation population.

"Prevent-extinction" procedure. – In my model, a given prey phenotype could not undergo a complete extinction. As a result of the prior procedures, a prey **behavioral strategy** could have temporarily gone to extinction at the previous procedure. Then, the program forcefully rescued the situation by generating a new prey individual with the extinct trait at the expense of a random existing individual. It was a design choice to prevent premature fixation and improve the robustness of the model outcome. With this procedure, the model could maintain the variation without any mutation or recombination; it helped me to minimize quantitative assumptions that are not directly related to my question.

"Predators-turnover" procedure. – A number of naïve predators entered the world and replaced some of the old predators. The user-adjusted variable **predator turnover rate** determined the proportion of the predators that were replaced by fresh ones, and the turnover occurred at the onset of the next prey generation. The new predators joining the model had zero **aversive memory**.

Final outcome: the winning strategy.

After 500 prey generations I determined the outcome of the simulation. The winning strategy was defined as the most abundant **behavioral strategy** among prey.

The settings used for this chapter.

ApoSim has many user-adjusted settings and variables (**Table 4.2**), and it was impractical to explore all the possible combinations in one focused study. Instead, I focused here on a subset of questions arising from the existing literature as outlined in the **Introduction**. I asked how several types of costs that prey incurs affect the evolutionary outcome, and how does the outcome depend on the learning speed and the influx of naïve predators into the system.

I decided to vary only five variables, as five-dimensional data would be close to the limit of meaningful visualization. I chose three variables relevant to general costs of signaling: **basal detectability**, **signal penalty**, and the **penalty of switchable signals** (which was composed of **switching cost** and **switchability maintenance cost**). I used two independent variables representing the properties of the predators: **predator turnover** and **learning speed**. Note that technically I varied 6 variables, but the **switching cost** and **switchability maintenance cost** were similar in nature, and I decided to co-vary them assuming positive correlation (which may not be true in some natural systems).

The combination of these five independent variables led to 29 376 different conditions, and I repeated the runs three times in each condition. This resulted in 88 128 outcomes in total. Besides these five independent variables, I treated every other user-adjustable variable as a controlled variable; I gave a fixed, reasonable value for each of them (**Table 4.2**). I want to emphasize that a future user of this software can choose completely different sets of independent variables.

Due to this incomplete search of the parameter space, my model result

should be interpreted with caution. My choice of control variables (**Table 4.2**) is supported by parameter-bracketing results (**Supplementary Figure 4.3**); however, it is still a set of arbitrary assumptions that should not be directly related to quantitative measurements from the real world.

Among the variables shown in **Table 4.2**, the list of **behavioral strategies** needs more clarification. As I mentioned in the first section of **Methods**, one of my core ideas is that the user can describe a variety of **behavioral strategies** in the three-letter notation.

Out of 27 possible combinations, I chose 9 strategies for this study based on the following assumptions. First, the prey can have only one switching opportunity at maximum. Therefore, the strategy can be either one of the pre-attack or post-attack switching, but not both. This was to aid visualization by making the competitive alternatives highly contrasting; compromised intermediates can be difficult to plot and describe. Second, I assumed that the increasing threat level can only associate to increasing levels of the **signal intensity**; the prey should stay at the current level or switch to a higher signal when freshly "approached" or "attacked." In the current version of the software, the predator detection check is only done once. This limitation made it useless to hide after initial interaction, because such behavior cannot alter the outcome of that encounter. With these two restraints, the possible combination of strategies reduced to 9 as seen in **Table 4.2**.

4. 3 Results

Two-dimensional mini-plots (**Figure 4.2**) are combined in three-dimensional meta-plots (**Figure 4.3**) that contain the outcomes of 88 128 model runs. Here, I briefly

describe the resulting **Figure 4.3**, highlighting comparisons among the figure elements that later in **Discussion** will be invoked again. From now on, I will regard **Figure 4.3B**, for the moderate **switching cost** and **switchability maintenance cost**, as the standard result analyzed in detail. I will then describe differences in **Figure 4.3A** or **4.3C** compared to **Figure 4.3B**.

In **Figure 4.3B**, NNH, NHH, or HHH **behavioral strategies** generally appeared in the moderate **learning speed** range. Conditions with extremely slow-learning predators favored NNN, the none-signal strategy (e. g. "**a"** of **Figure 4.3B**). As the **learning speed** increased, strategies with high **signal intensity** began to appear (e. g. the transition from "**a**" to "**b**" of **Figure 3B**). The opposite extreme condition with fast-learning predators also favored NNN (e. g. "**d**" of **Figure 4.3B**). There was a tendency of smooth tapering from high- to low- and finally to no-signal strategies as the **learning speed** increased (e. g. the transition from "**e**" and "**f**" columns to "**d**" column in **Figure 4.3B**).

Conditions with higher **basal detectability** and higher **learning speed** selected the pre-attack or permanent signaling strategies, while lower **basal detectability** and lower **learning speed** favored post-attack strategies (e. g. contrasts between "**b**," "**c**" and "**f**" in **Figure 4.3B**). If the **basal detectability** was extremely high while the predator **learning speed** was extremely slow, then there was no stable winner, and the outcome was mostly random (e. g. "**g**" of **Figure 4.3B**).

If **learning speed** and **basal detectability** were both in the moderate range, the **signal penalty** dominated the performance of **behavioral strategies**. The lower **signal penalty** led to stabilization of permanent signals; higher **signal penalty** favored post-attack signals; pre-attack signals won in the middle ground (e. g. "**h**"

of **Figure 4.3B**).

Finally, if the **predator turnover** was high, the influence on the outcome appeared mostly identical to the slowing down of the effective **learning speed**. For example, in **Figure 4.3B**, the shift from "**e**" to "**b**" (decrease in **learning speed**) was similar to the shift from "**e**" to "**i**" (increase in **predator turnover**.) However, when the **learning speed** was high, the **predator turnover** rate was a determining factor for the emergence of the post-attack signals: if the turnover was low, higher **learning speed** conditions mostly selected permanent or no signals strategies (e. g. "**k**" of **Figure 4.3B**); if the turnover was high, post-attack signals were also selected (e. g. "**l**" of **Figure 4.3B**).

Figure 4.3A shows the corresponding set of results with no fixed or per-use costs in switching signals (i.e. **switching cost** = 0 and **switchability maintenance cost** = 0; **Table 4.2**). In this condition, permanent signal strategies disappeared almost entirely, and the switching strategies replaced them. Another difference is that the post-attack switching strategies became more common in normally unfavorable, extreme conditions.

Figure 4.3C shows results for situation in which the switching phenotypes pay very high costs (i. e. **switching cost** = 0.016 and **switchability maintenance cost** = 0.000255; **Table 4.2**). The figure shows that the no-signal strategies replaced most of the switching strategies. The switching phenotypes survived only in the narrow zone of moderate **learning speed**, high **basal detectability**, and extremely high **signal penalty**.

To assess the robustness of the result, I have run simulations equivalent to **Figure 4.3** with different levels of control variables (**Supplementary Figure 4.3**). Readers should be aware that however reasonable they are, my choices of control

variables and their values are strictly arbitrary. Furthermore, as apparent in the **Methods**, the model structure is an abstract and simplified simulation, not a faithful reconstruction of a complex natural system. Therefore, the numerical values form the model should be interpreted as generic tendencies, rather than exact quantitative predictions of relationships that could be empirically discoverable in the real natural systems.

4. 4 Discussion

The model results (**Figure 4.3**) help in understanding evolutionary transitions between various forms of aposematic adaptations in prey. For instance, a defended prey may undergo evolutionary transitions from NNN (non-signaling) to NHH or NNH (examples of switchable aposematic signals), from NNN to LLL or HHH (permanent aposematism), or from HHH to NNH or NNN. my model visualizes how such evolutionary transitions may be driven by changes in ecology of predators and prey. Here, I will first discuss in details the conditions that favor specific anti-predatory adaptations by focusing separately on each of the five independent variables (**Table 4.3**). Then I will discuss possible evolutionary scenarios towards switchable aposematic signals. Finally, I will also look closer at some of the existing and novel hypotheses about the evolution of various forms of aposematism and how the model may contribute to understanding of their evolution. To achieve clarity and precision of my presentation, here I refer by number to relevant figures and tables in the **Results**.

Predator learning and population turnover.

The results are consistent with the existing theoretical coverage of the effects of predator learning and forgetting on prey aposematic signals (Speed 2001). For example, some of my model runs included predators whose learning speed is so extremely slow that the aposematic signals become useless. This condition is seen in "**a**" of **Figure 4.3B**. Increase in the predator **learning speed** eventually leads to the relatively abrupt appearance of the strongest possible signals (H), be it switchable (**Figure 4.3A, B**) or not (**Figure 4.3C**; here the permanent signals are less costly). One such pattern is visible in the transitions from "**a**" to "**b**" in **Figure 4.3B**. This suggests that there may be a threshold of predator **learning speed**, below which aposematism (switchable or permanent) cannot outcompete the cryptic forms. This is because the prey cannot give infinitely effective signal while the predator **learning speed** can reach near zero; below some point of learning speed the prey should fall back to crypticity instead of investing more into the signal. When the predator **learning speed** is barely above this crypticity-aposematism threshold, strong signals (H) are observed (e. g. "**b**" or "**c**" of **Figure 4.3B**) because high signal strength is needed to cause efficient learning in the relatively slow learning predators.

As seen in **Figure 4.3B**, the crypticity-aposematism threshold is highly dependent on the **predator turnover** and **basal detectability**. **Predator turnover** determines the benefit of aposematism, as it determines the residence time of the educated predators; **basal detectability**, the baseline chance of being discovered, determines the benefit of crypticity.

Slightly above the crypticity-aposematism threshold, the post-attack aposematism frequently dominated the runs (e.g. from "**a**" to "**b**" in **Figure 4.3B**). When predators learn slowly there is higher chance of encountering a predator with little

knowledge, and it is more likely to be attacked upon discovery. Under this condition, post-attack strategies, which minimize the risk of detection, should be favored if switching-related costs are not too high (**Figure 4.3A, B**).

On the other extreme of learning speed, my model shows an alternative situation. Under very high **learning speed** (e. g. "**d**" of **Figure 4.3B**), NNN strategies are the most successful ones. In my model, predators can learn from the prey **basal cognitive cue** even when no signal is given from the prey. If **learning speed** is high enough, this basal learning can grant adequate protection. Hence, in this situation the **basal cognitive cue** fulfils aposematic function; it should be noted that the phenotype NNN is actually protected by aversive learning unlike the situation at the slower extreme of **learning speed**. It is possible that some fast-learning predators may indeed not need any auxiliary signal from the prey in order to recognize it as defended. For instance, it has been demonstrated that oriental tits can learn the basic, inconspicuous morphological features of an insect as cues of unprofitability, albeit at a slower rate compared to learning of a typical aposematic signal (Kang et al. 2016a).

The transition toward this 'silent' form of aposematism appears to be more gradual (e.g. from "**m**" to "**d**" in **Figure 4.3B**) compared to the relatively abrupt transition between NNN and signaling strategies (e.g. from "**a**" to "**b**" in **Figure 4.3B**) at the slower **learning speeds,** which I have already discussed. This effect of a very high learning rate could be inferred from earlier models (Speed 2001; Puurtinen & Kaitala 2006). Especially, the gradual transition toward weaker conspicuousness fits well to the situation described in seasonal predator psychology studies (Endler & Mappes 2004; Mappes et al. 2014).

The effect of the decreased **predator population turnover** largely

corresponds to the effect of increased **learning speed**. As a result, the entire 3-dimensional plot is compressed/shifted to the right side (requiring higher **learning speed**) as **predator turnover** increases. This is because the influx of naive predators hinders the collective memory build-up in the predator population, and the average predator behavior for a given prey becomes similar to what can be expected in slower-learning but lower-turnover predator population.

However, the high **predator turnover** leads to a unique outcome with the very fast-learning predators that can associate prey defense with the prey **basal cognitive cue**. As apparent in "**l**" of **Figure 4.3B**, which can be contrasted to "**k**" of the same figure, high **predator turnover** favors the evolution of weak post-attack aposematism when the predator **learning speed** is sufficiently fast. In this scenario, the predator knowledge level is highly variable (because of the high turnover), and the prey cannot know if the approaching predator is a naive or experienced one. Therefore, the post-attack signal, which would be useless if the predator population was full of well-educated predators, is still needed to educate and deter the fresher portion of the predator population.

The mix of experienced and naïve predators has been a popular topic in studies of permanent aposematism (Lindstrom et al. 1999; Speed 2001; Svadova et al. 2009). Especially, the studies on the seasonal variation of predator knowledge (Endler & Mappes 2004; Mappes et al. 2014) provide highly advanced analyses about the continuous influx of naïve predators and the need to repeatedly educate them. It was suggested that the post-attack display of the spotted lanternfly, *Lycorma delicatula*, is an adaptation to this circumstance (Kang et al. 2011; Kang et al. 2016a). The results of my simulations indeed confirm that the weak post-attack switchable aposematism is actually the best strategy to deal with the mix of

naïve and experienced predators of high learning speed.

Basal detectability.

Empirical and theoretical studies about deimatism (startle display) and post-attack defense have already discussed the general effect of the **basal detectability** (Broom et al. 2010; Willink et al. 2013; Umbers et al. 2015). However, to my knowledge, there is no currently available literature that focuses on evolution of switchable aposematism while overviewing the importance of the **basal detectability** along with other driving forces.

My results are consistent with the general prediction that low **basal detectability** would favor the post-attack aposematism (e.g. panel "**b**" in **Figure 4.3B**) rather than the pre-attack or the permanent aposematic signals (e.g. panel "**c**" in **Figure 4.3B**). Initiation of an attack by a predator is a clear indication that the prey has been spotted. But before that moment of attack there is always a chance that the approaching predator has not detected the prey, especially for prey with low **basal detectability**. In this situation, it would not be beneficial to the prey to use a pre-attack display because such a behavior would reveal its presence to the predator, who otherwise is unlikely to be aware of its location. Similarly, the prey with permanent aposematic signals would entirely lose the opportunity to benefit from the potential crypticity. On the other hand, the post-attack signals are only emitted when the predator has already detected the prey and decided to attack (Broom et al. 2010). In such a scenario, giving post-attack aposematic signal cannot increase the risk of detection, as it is already detected. Hence, if the prey can energetically and ecologically afford the brief display of the signal, the post-attack switch-on can only help the prey, not harm it (unless the predator is more

intrigued by the signal received during the attack). Therefore, post-attack signals can be a viable strategy in the conditions that are not favorable to pre-attack or permanent alternatives.

Higher **basal detectability**, on the other hand, effectively lowers the opportunity cost of aposematism. As an approaching predator is likely to detect the conspicuous prey anyway, the pre-attack or permanent aposematism would not bring much additional risk. As discussed before, the low **learning speed** is a condition where post-attack signals generally prevail. However, the high **basal detectability** and the reduced opportunity cost can bring the balance toward pre-attack/permanent aposematism (transition from "**b**" to "**c**" of **Figure 4.3B**). If the additional risk is negligible, pre-attack or permanent aposematism offer faster memory retrieval which grants the prey further protection.

If the predator **learning speed** is too low in this condition, then the signal is void of both risk and benefit - it may not significantly add to either detection risk or predator deterrence. In this situation, the selection pressure on aposematism-crypticity axis is largely absent, and no specific strategy would be clearly favored (e. g. "**g**" of **Figure 4.3B**) under my set of assumptions.

Costs of signals.

The general effect of the various types of costs on the evolution of post and pre-attack defenses have been analyzed earlier (Broom et al. 2010). However, to my knowledge, there had been no overarching theoretical perspective that shows the full implications of these costs when faced with learning-capable predator population. my results provide a solid background to discuss the interactions

between costs of a signal and costs of its switchability pertaining to the evolution of aposematism.

The **signal penalty** is a value that represents the energetic cost of the signal as well as the exposure to the potentially signal-unfriendly environment (**Table 4.3**). If other variables have moderate values, the increase in **signal penalty** leads from permanent to pre-attack, and to post-attack aposematism (as seen in "**h**" of **Figure 4.3B** and other similar mini-plots). As the signal is given for shorter and shorter duration along the above sequence, it is the logical order to constrain the increasing **signal penalty** cost into an affordable range. However, as seen in most of the other mini-plots in **Figure 4.3B**, one should note that if other variables do not favor certain forms of aposematism, the **signal penalty** alone - even when extremely high or low - is insufficient to promote all possible types of aposematism.

Figure 4.3A shows the model outcomes when **switching cost** and **switchability maintenance cost** (together representing the "penalty of switchable signals") are absent while **signal penalty** is present. In this condition, the permanent signals would be generally inferior to the switchable alternatives because of the longer duration of unnecessary exposure. Without any additional cost, the switchability provides finer control over the **signal intensity** fitting the circumstances. Therefore, the permanent aposematism present in **Figure 4.3B** mostly disappeared in **Figure 4.3A**.

Though less pronounced than in the permanent signals, some difference in the post-attack signal is also visible in **Figure 4.3A**; the post-attack signals are more likely to stabilize in signal-unfriendly extremes. Being switchable, post-attack signals have two types of costs – the **signal penalty** (cost associated to the **signal intensity**) and the switching-related costs (the fixed **switchability**

maintenance cost and the per-use **switching cost**). Considering that the post-attack signal is presented only after a predator attack, the **signal penalty** would be largely avoidable during the most of prey lifetime. Therefore, the reduction of switching-related cost could substantially alter the cost-benefit balance of this strategy, which can be seen from the difference between **Figure 4.3A** and **4.3B**.

Figure 4.3C shows the other end of the spectrum. It depicts the model outcome when the signal-switching behavior and the maintenance of switchability are very costly. Naturally, almost all switchable signals disappeared, and permanent aposematism became more common. In comparison to **Figure 4.3B**, one can see that the pre-attack signals mostly changed to the permanent signals while post-attack signals generally reverted to permanent none-color strategy. Permanent signal is a good substitute for pre-attack signal except for the lengthened display duration; in predator deterrence, they serve essentially the same purpose. On the other hand, the stability of the post-attack aposematism is mostly due to the utilization of crypticity while not being attacked. Therefore, if the switchable signals are not an option due to high costs, the post-attack strategy is replaced with the no-signal strategy that maintains the benefits of crypticity.

Conditions for evolution of switchable signals.

Based on the model results, I hypothesize that the relative values of the two types of cost, the **signal penalty** and the **penalty of switching**, predict the evolution of switchable or permanent signals (**Figure 4.4**). As the costs of switchable signals increase from **Figure 4.3A**, **4.3B** to **4.3C**, the switchability-favoring range of signal penalty narrows down (e. g. from the whole range in panel "**o**" in **Figure 4.3A** to

none in in panel "**p**" o, I deliberately configured the software to exclude any startling ('deimatic') or learning facilitation effects of the switched signals. In the setup used in this paper, the strength of the signal being switched on is the only relevant factor in predator psychology; the fact that it was switched does not have any effect except that it imposes some cost to the prey. This decision helped me to study the cost-saving aspects of switchable aposematism without further complications. However, this assumption is far from reality (Umbers et al. 2015; Kang et al. 2016a; Ruxton et al. 2018), and the model predicts that the switchable aposematism would be more favored if startle and facilitation effects could provide survival advantages (**Supplementary Figure 4.1**).

Visualizing hypothetical evolutionary pathways leading to aposematism.

Although my model is not designed to directly imitate predator-prey coevolution, its results (such as **Figure 4.3**) can provide a useful aid to contemplate the evolutionary effect of changes in environment and in predator ecological guild. **Figure 4.5** shows how such changes may mediate a variety of possible evolutionary pathways to different forms of aposematism in the defended (unprofitable) prey.

Scenario "a": highly detectable prey invokes learning in predators - The path "**a**" of **Figure 4.5** represents a hypothetical scenario starting from non-cryptic and non-aposematic prey phenotypes. The ancestral non-crypticity (high **basal detectability**) could have emerged by chance, or due to selective processes other than aposematic signaling. Examples of such conditions include sexual selection and conspicuous

behaviors such as flight. my model predicts that such traits would affect the existing predation pressure very little if **basal detectability** is high and the **learning speed** is slow (see **Basal Detectability** section of **Discussion**). Once the defended prey has acquired those non-cryptic unique characteristics that are different from the undefended ones in the same ecosystem, a selection pressure to distinguish them may apply to the predators. This can lead to an increase in predator **learning speed**, which may in turn favor signaling strategies in prey, thereby initiating a true aposematic interaction.

This scenario may be considered in sexual selection context (Maan & Cummings 2009; Crothers et al. 2011; Ruxton et al. 2018), because it provides a straightforward condition that can lead to highly conspicuous appearances before aposematic evolution occurs. Another important example regarding this pathway is the mobility benefit (Speed et al. 2010). If the prey is already conspicuously mobile due to the need in resource collection, this is a condition that highly favors the evolution of aposematism. my visualization in "**a**" of **Figure 4.5** is consistent with those two scenarios and expands them with the insights gained from other related variables (**Figure 4.3**).

Scenario "b": intelligent predators may provide a starting ground for evolution of aposematism - Path "**b**" of **Figure 4.5** illustrates a hypothesis that the ancestral predators have very high learning capability. In this scenario, the **basal cognitive cue** - the minimal uniqueness in body shape and behavior that arose from prey ecology – has been already performing an antipredatory aposematic function (see **Predator learning and population turnover** above). However, the variation in predator intelligence could have demanded some slight 'nudge' to help the

defended prey in being correctly discriminated. Once the prey evolves this minimal cognitive aid that increases its survival, then this frees up the predators from the burden of cognitive and behavioral investment that enabled the initial identification. This degradation of learning capabilities then could have begun the down-spiral toward highly conspicuous aposematic systems. In the final stages, as in the previous scenario, the relative ratio between signal penalty and switching penalty determines whether permanent or switchable aposematism is established.

Alternatively, the path "**b**" is also consistent with a scenario that does not assume co-evolution but simply represents a change of ecological guild of predators to which the prey is exposed: from extremely fast learners to intermediately fast learning predators. One can easily imagine that a prey invading new habitats may occasionally experience such changes. Similarly, a sudden or gradual change of predators' guild composition may shift the prey conditions from facing the "very fast learning" predators to facing "moderately fast learning" predators. By considering such ecologically driven gradual evolution of prey signals, the scenarios along pathway "**b**" directly provide alternative solutions to the controversy surrounding the possibility of gradual development of aposematism through co-evolution (Lindstrom et al. 1999; Endler & Mappes 2004; Ruxton et al. 2018).

Scenario "c": post-attack switchable aposematism as ancestral form of aposematism - It is reasonable that a prey at the post-attack stage would be more easily identified than at the pre-attack stage. After being attacked the prey would attempt to struggle or flee, revealing the prey's various body parts, behavioral peculiarities, and other information. Even if the predators have not used this

information yet, the prey might have been already under selection to intensify this post-attack behaviors in order to increase survival through escape. This may lead to more and more unique and conspicuous prey reactions, not because the conspicuousness was needed but as a byproduct of selection toward effective escape behaviors. It is feasible to assume that this intensification of post-attack behavior involves increase in its memorability to the predator. The higher memorability can effectively increase the learning speed, shifting the conditions from favoring "none-signal" (gray shaded panel in **Figure 4.5**) to those favoring post-attack signal (blue panels). Alternatively, the predators might evolve better cognitive ability (**learning speed**) to recognize the post-attack behavior of the defended prey because it may increase their foraging efficiency. Either way or both, my model predicts that the shift in the **learning speed** would lead to a new selection regime that will drive strong post-attack aposematism.

An alternative version of the above process may involve a shift from ecological conditions favoring no signaling (gray panels in **Figure 4.3**) to conditions favoring post-attack aposematism (blue panels) due to a decrease in the influx of naïve predators (decrease in the **predator turnover** rates). This can be seen in **Figure 4.3B** where panels in the lower left corner (low **learning speed**, low **basal detectability**) change from gray (no signals) to blue (post-attack signals) as the predator turnover decreases.

After post-attack aposematism is established for any reason, the pressure to maintain prey crypticity (i. e. low **basal detectability**) can be relieved if the strategy provides sufficient protection. In this condition the prey may become more easily detectable, and this trend may be associated with the increase in prey memorability through the heightened **basal cognitive cue** and the encounter

frequency. The increased memorability is a boost to **learning speed** by itself, and it may also trigger natural selection for faster learning; the cognitive investment may now bring more benefits in foraging efficiency. The increased learning speed may create a situation when the post-attack is no longer the evolutionarily winning strategy. Instead, depending on the ratio of switching penalty relative to signal penalty, the pre-attack or permanent aposematism would be favored. In summary, this scenario shows that the post-attack aposematism could open evolutionary pathways to other forms of aposematism. This hypothesis is similar to an existing theory about evolution of aposematism in physically defended animals (Speed & Ruxton 2005) except that my focus is on behavioral reactions rather than on the physical devices.

Model assumptions and limitations.

The above discussions illustrate how my model may contribute to research on diversity of aposematic strategies. However, in order to draw proper generalizations and use the model to answer new questions, one needs to understand the model's assumptions and limitations.

First, my model does not feature any coevolution, neither between species nor between traits. Predators can only learn and do not evolve over generations; the prey **behavioral strategy** is the only trait that has variation for natural selection. In nature, one can expect that there would be predator-prey coevolution as well as the coevolution between behavior, morphology, and physiology. However, proper modeling of such evolutionary interactions would require a number of quantitative assumptions that would limit the applicability and usability of the software.

Considering that the signaling **behavioral strategy** is already a multi-dimensional variable, I found that the further complexity in the modeled world give little benefit while immensely interfering with the visualization, hypothesis formation and the interpretation of reality using the model. Instead, by keeping constant the components that are reasonably expected to evolve slower than prey **behavioral strategy**, I were able to obtain a complete view across a wide range of conditions. my primary goal was to generate clear predictions about optimal prey **behavioral strategies** in a given combination of environmental variables and predator phenotypic and population traits. I realized that modeling coevolution, albeit intriguing, could complicate the accomplishment of this goal.

One omitted variable, however, deserves a more detailed discussion: the prey defense. my model treats the prey defense as a given fixed value for each prey individual (**repulsive taste**), not allowing for variation among prey individuals. It could be seen as an unrealistic assumption because the defensive capability and anti-predatory communication ability is viewed as a closely interacting pair in some systems (Sword et al. 2000; Sherratt 2002; Broom et al. 2005; Speed et al. 2006).

However, allowing the evolution of repulsive taste in my model gives unnecessary complications with minimal benefit. Since the **repulsive taste** gives one-sided benefit in context of predator deterrence, simply allowing this component to vary is not a proper way of modeling its evolution. It must be accompanied by properly simulated mechanisms of defense cost, which need to bring in an array of assumptions about prey physiology and anatomy, as seen in the theories and discussions introduced above. Furthermore, prey defense can evolve without help of aposematism; chemical, mechanical, or behavioral qualities for

antipredator defense could be beneficial in many other contexts as well. This complexity of the real world adds to the difficulty of expanding my model by including defense evolution and the associated mechanisms of cost-benefit tradeoffs of defense. Therefore, in my model I decided to set the prey defense to a constant value set by the user (**repulsive taste** value). I hope that the achieved simplicity and universality of the model may be viewed as a strength for asking specific questions that focus on evolution of various signaling strategies.

I also viewed another omitted variable, the common maintenance cost of both the permanent and switchable signals, as a similar unnecessary complication. Unlike the signal 'switchability' maintenance that needs to be balanced against the dynamic benefit of the switchability, the static signal maintenance is a common penalty for both switchable and permanent aposematism. This had lower priority within my primary purpose: my question was focused on the evolution of the switchable aposematic signals, not the question of aposematism versus non-aposematism. For readers who are interested in the detailed mechanisms governing the latter type of evolutionary balance, I would like to recommend other existing theories over ours; Mappes et al. (2005) and Ruxton et al. (2018) could serve as good entry points.

Finally, for the purpose of this particular paper, I deliberately configured the software to exclude any startling ('deimatic') or learning facilitation effects of the switched signals. In the setups used in this paper, the strength of the signal being switched on is the only relevant factor in predator psychology; the fact that it was switched does not have any effect except that it imposes some cost to the prey. This decision helped me to study the cost-saving aspects of switchable aposematism without further complications. However, this assumption is far from

reality (Umbers et al. 2015; Kang et al. 2016a; Ruxton et al. 2018), and the model predicts that the switchable aposematism would be more favored if startle and facilitation effects could provide survival advantages (**Supplementary Figure 4.1**).

Closing Remarks.

In summary, my simulation provided support for many hypotheses about the diversity of aposematic strategies, especially focused on signal switchability. These include, but are not limited to, the following insights. First, the evolution of pre-attack switchable aposematism may require moderate range of predator learning speed, high basal detectability of the prey, and moderate to high level of signal penalty. Second, the post-attack switchable aposematism may be favored under relatively low level of predator learning, low prey basal detectability, high signal penalty. Third, high predator turnover combined with fast learning speed, a condition which produces a mix of naïve and experienced predators, may facilitate evolution of post-attack aposematism. Fourth, higher cost related to signal switching may lead to disappearance of post-attack aposematism while forcing pre-attack aposematism to be fixed and permanent. During my study, I developed an individual-based modeling framework that can be used to explore a variety of questions regarding aposematism. This product is capable of simulating a number of variables that are not covered in this article, and I hope that it will inspire scientists and educators to further study the evolutionary biology of aposematic signals.

Table 4.1. The modeled predator-prey interaction timeline.

prey generation	interaction frame	time step	program procedures
gen. 1	frame 1	step 1	predators-move
			prey-react
			prey-pay-cost
			predators-search
			predators-observe
			predators-analyze
			predators-decide
		step 2	predators-initial-act
			predators-feel
			prey-react
			prey-pay-cost
			predators-observe
			predators-learn
			predators-analyze
			predators-decide
		step 3	predators-final-act
			predators-feel
			prey-react
			prey-pay-cost
			predators-observe
			predators-learn

frame 2
…
frame 50

(repeat the three time steps for each interaction frame)

reproduction stage

prey-selectively-die
prey-lay
all-prey-die
prevent-extinction
predators-turnover
prey-emerge

gen. 2
…
gen. 500

(repeat the 50 interaction frames and the reproduction stage for each generation)

Table 4.2. The list of model parameters and the values used in this chapter.

parameter	value(s)
CONTROLLED VARIABLES†	
world size	14
generation length	50
prey frequency	92%
predator frequency	23%
low-signal (L)	0.3
high-signal (H)	1
initial attack damage	0.1
final attack damage	0.4
basal cognitive cue	0.1
repulsive taste	0.1
behavioral strategies	[NNN, NNL, NNH, NLL, NHH, LLL, LLH, LHH, HHH]
INDEPENDENT VARIABLES	
switching cost††	"none" (0), "moderate" (0.0005), "high" (0.016)

switchability maintenance cost††	"none" (0), "moderate" (2.03E-4), "high" (0.00340)
basal detectability	0.05, 0.15, 0.4, 0.8
predator turnover	0.01, 0.25, 0.7, 1
learning speed	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300, 1000
signal penalty	0.13, 0.1105 -- ratio 0.85, geometric sequence -- 5.32E-5, 4.52E-5, as well as 0

† Not all controlled variables are explained in the main text, as some of them are not relevant to the questions asked in this chapter. For the full description of all simulation parameters, please see **Appendix**.

†† For the purpose of this chapter, the two variables were treated as one set under the name 'penalty of switchable signal,' and they changed simultaneously between three states: none, moderate, or high.

Table 4.3. The independent variables used in the chapter and the corresponding natural examples.

variables	short definition	examples (low level)	examples (high level)
COSTS TO PREY			
basal detectability	basal discovery chance when not giving a signal	**dead leaf mantis** nearly impossible to discover when not doing threat display	**stotting ungulates** their size and habitat often make them highly noticeable even when not leaping
signal penalty	inherent cost associated to higher level of signal	**skunk body stripes** there is little evidence of disadvantage for more striking bands	**black widow spider** the red dorsal mark is selected to be moderate because it can be seen by prey insects (Brandley et al. 2016)
switching cost	cost paid per each switching behavior	**color under wings** half a wingbeat to expose pigmentation is very cheap compared to regular flight	**fire-bellied toad unkenreflex** the complete flip of the body is a big, energetic movement that may hinder many other useful behavior

switchability maintenance cost	cost of maintaining the ability to switch signals	**color under wings** if flight is maintained anyway, there is almost no additional cost to switchability	**rattlesnake rattle** fragile structure that is kept lifted in locomotion; the tail tip cannot be used in versatile activities like other snakes

PROPERTIES OF PREDATORS

learning speed	coefficient of aversive memory buildup	**mantis-milkweed bug** many intermediate stages before reaching complete aversion (Gelperin 1968)	**primate-snake** aversion acquired quickly and intensely (Ohman & Mineka 2003)
predator turnover	proportion of naïve predators entering per prey generation	**blue jay-monarch butterfly** every year only a fraction of the population needs to learn about cardenolide	**mantis-milkweed bug** probably the whole annual population needs to freshly learn the aversion (Gelperin 1968)

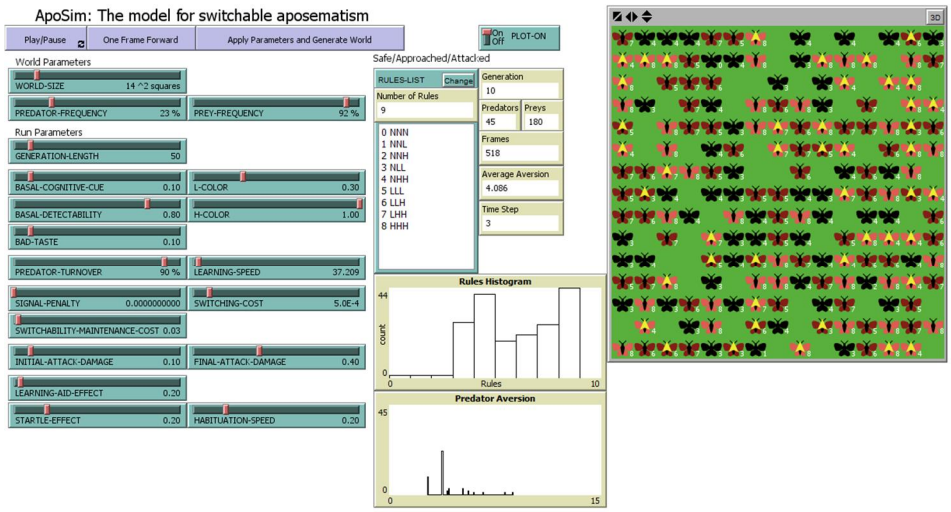


Figure 4.1. The ApoSim model interface built with NetLogo.

The model interface is divided into three parts. In the leftmost panel, the user can adjust model variables using sliders and input windows. In the middle panel, the model status is reported in various plots and displays. In the right panel, the animated view of the two-dimensional world is shown.

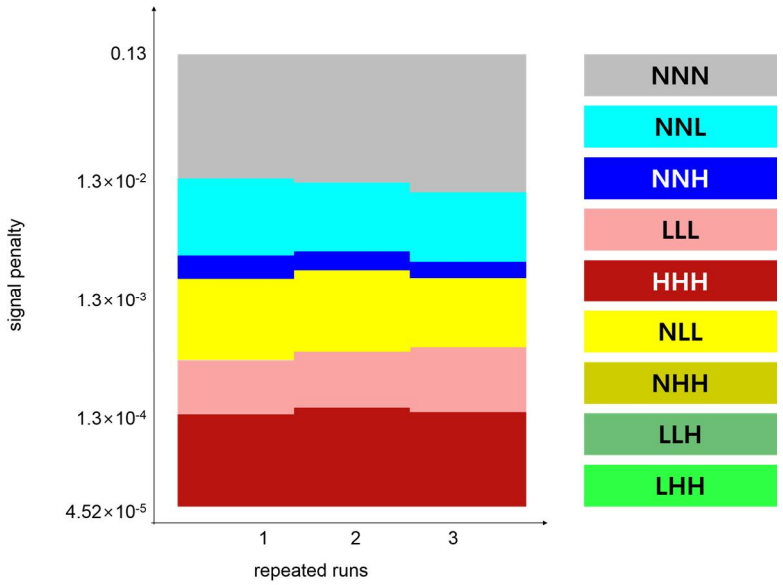


Figure 4.2. An example mini-plot of the model results and the color codes for the behavioral strategies.

The prey **behavioral strategy** that won each run is depicted according to the color code. For the three-letter annotation of the **behavioral strategies**, see the first section of **Methods**. The vertical axis is the **signal penalty** value used in each run. There were three repeated runs for a given combination of conditions, and these repeats appear along the horizontal axis. Therefore, a mini-plot visualizes the result of 153 runs in total, with 51 **signal penalty** values and 3 repeats. For more detailed information about the run parameters, see **Table 4.2**. Mini-plots like the one shown here are the building blocks that constitute the meta-plots in **Figure 4.3**.

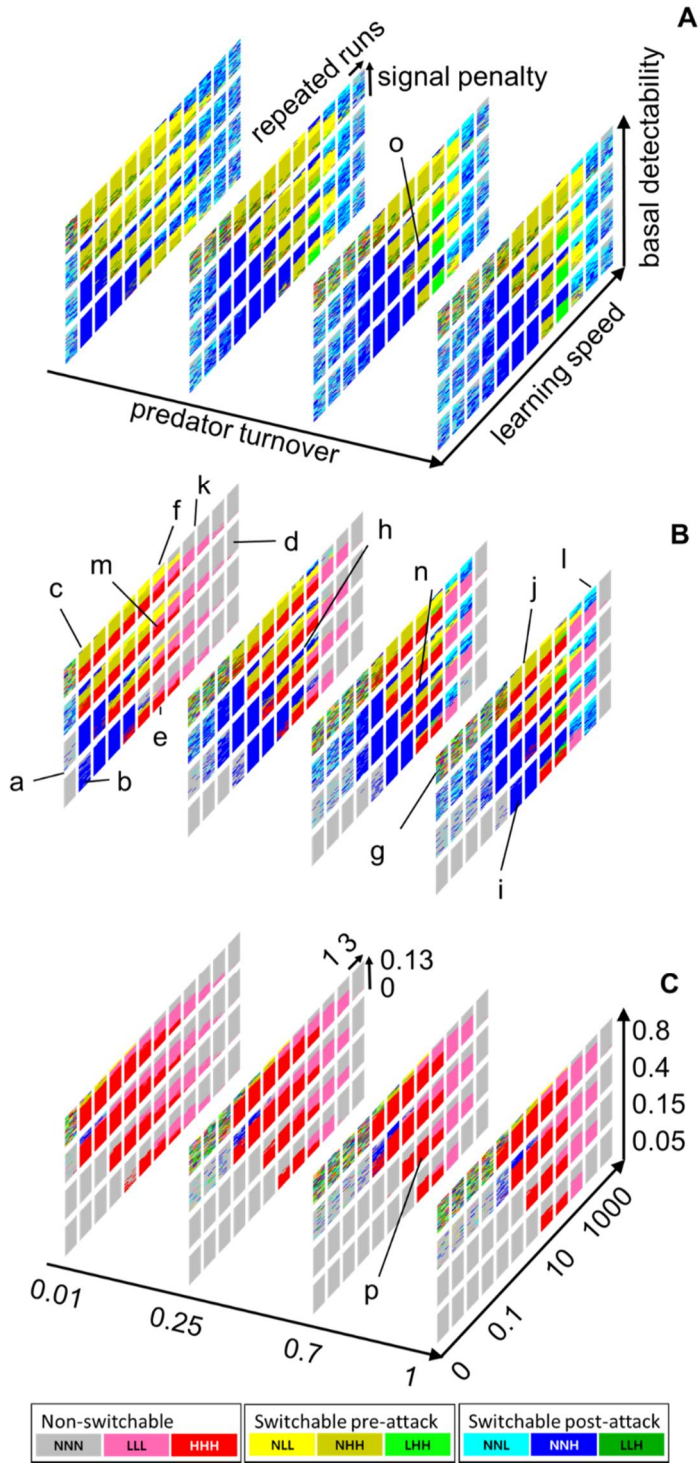

A
repeated runs
signal penalty
basal detectability
predator turnover
learning speed
o
B
k
f
d
h
m
c
n
l
j
e
a
b
g
i
C
3
1
0.13
0
0.8
0.4
0.15
0.05
0.01
0.25
0.7
1
0
0.1
10
1000
p
Non-switchable
NNN
LLL
HHH
Switchable pre-attack
NLL
NHH
LHH
Switchable post-attack
NNL
NNH
LLH

Figure 4.3. Model result meta-plots.

The winning **behavioral strategies** over the entire range of the tested parameters given in **Table 4.2**. For the color code and the interpretation of each mini-plot, see **Figure 4.2**. The x-axis, the one that runs from left to right, is the predator **learning speed**. The y-axis is the **predator turnover rate**. The z-axis is the **basal detectability** of the prey. For more detailed information about each variable, see **Methods** section. A, the **switching cost** and the **switchability maintenance cost** are both none; B, there are moderate costs for both; C, the costs are prohibitively high. For more detail about the three levels of penalty of switchable signals, see **Table 4.2**. Note that in order to avoid the visual clutter in the three-dimensional view, I labeled each subpanel (A, B, C) with different sets of information; the names of the axes, the regions of interest in lowercase letters, and the values of axes are separately given in subpanel A, B, and C, respectively. However, all notations are applicable to all three subpanels. Regions of interest indicated by small letters (a – p) are referenced in the main text of the **Results** and **Discussion** sections.

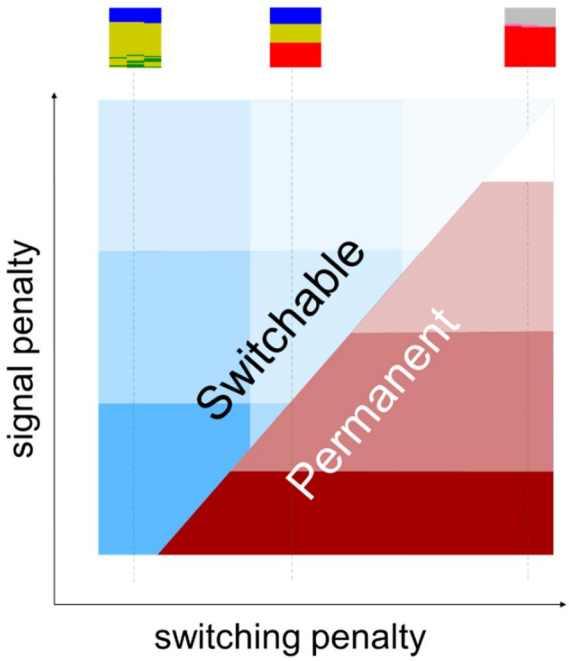


Figure 4.4. A two-dimensional diagram for evolutionary stability of permanent versus switchable aposematism.

This is an evolutionary "phase diagram" to abstractly express the stability of the two modes of aposematism. The horizontal axis is the signal penalty, and the vertical axis is the switching penalty (a collective representation of both per-use switching cost and the fixed switchability maintenance cost). The reddish-brown color represents the strength of permanent signals that are expected to be stable in the given condition. The blue color represents the strength of the switchable signals in general, without distinguishing pre- from post-attack switching. Both colors fade as the costs increase indicating that neither permanent nor switchable displays are likely to evolve for extremely high values of both costs. Inlets are mini-plots "**n**," "**o**," and "**p**" of **Figure 4.3**, reproduced as examples of three switching penalty levels. For the color codes, axes and ranges of the mini-plots, please see **Figure 4.2**

and **Figure 4.3**.

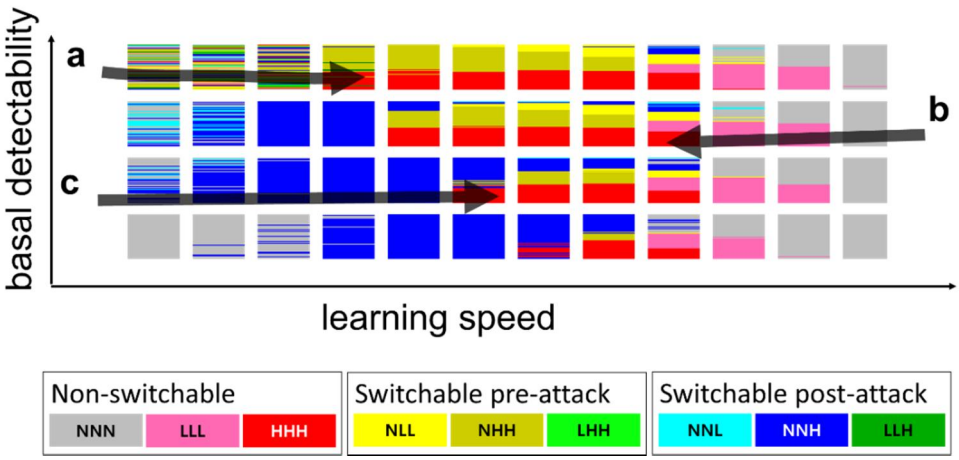
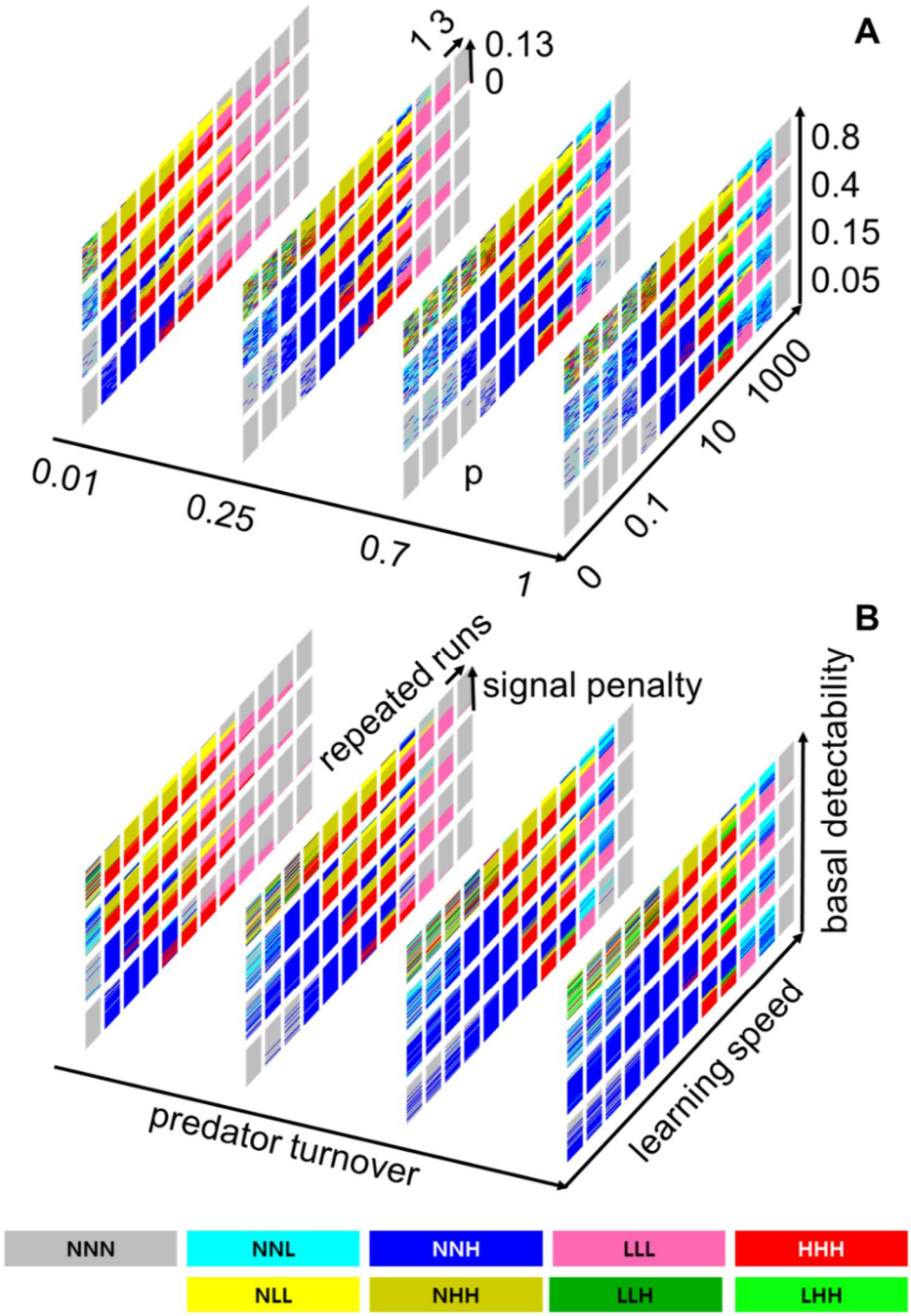


Figure 4.5. Three hypothetical pathways towards aposematism.

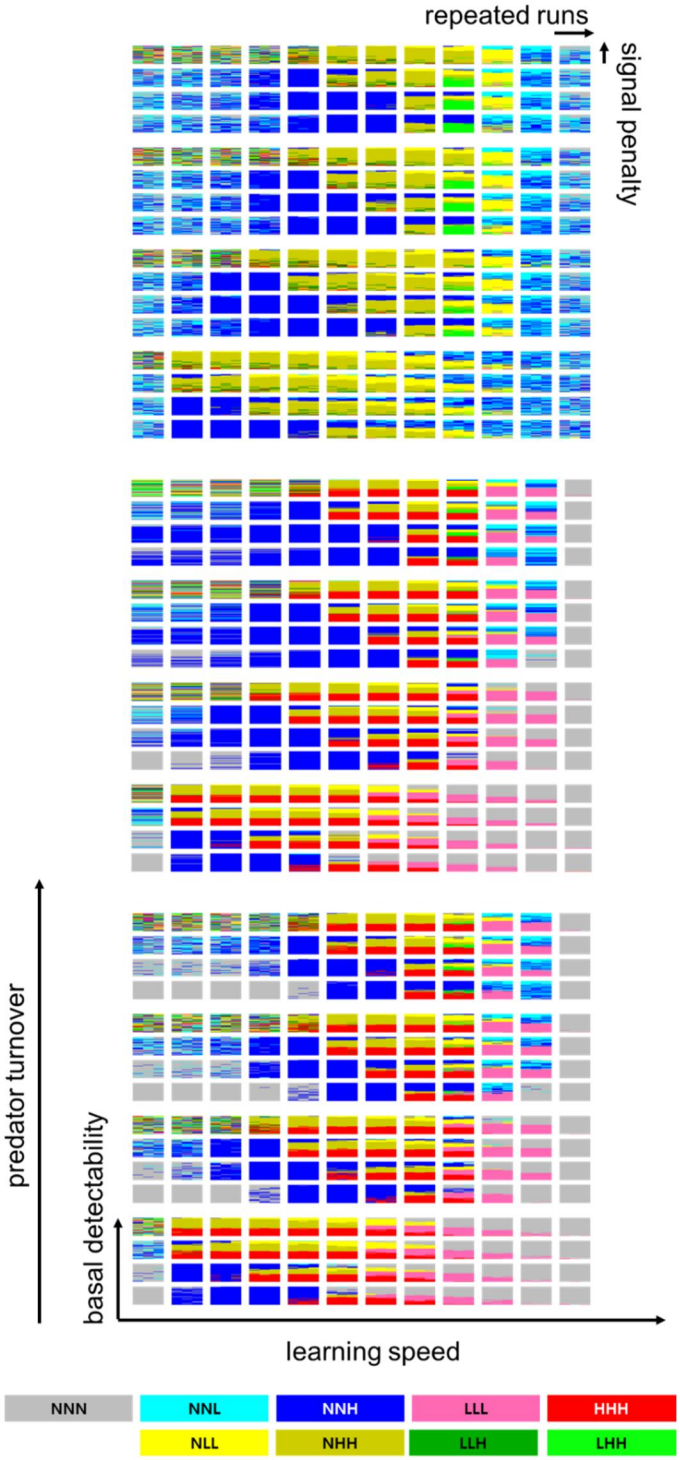
A portion of the model outcome meta-plot in **Figure 4.2** is shown again for explanatory purpose. For the color code and the interpretation of each axis and mini-plot, see **Figure 4.1** and 4.**2**. The labeled arrows **a**, **b**, and **c** illustrate the hypothetical pathways, and each is referenced in the main text. These are examples of how the model can be used to discuss hypothetical evolutionary pathways between various forms of (non-)aposematism. Hypothetical mechanisms and ecological conditions critical along such pathways can be identified using the software.

4. 5 Appendix


A
1.3
0.13
0
0.8
0.4
0.15
0.05
0.01
0.25
0.7
1
p
0
0.1
10
1000
B
repeated runs
signal penalty
basal detectability
predator turnover
learning speed
NNN
NNL
NNH
LLL
HHH
NLL
NHH
LLH
LHH

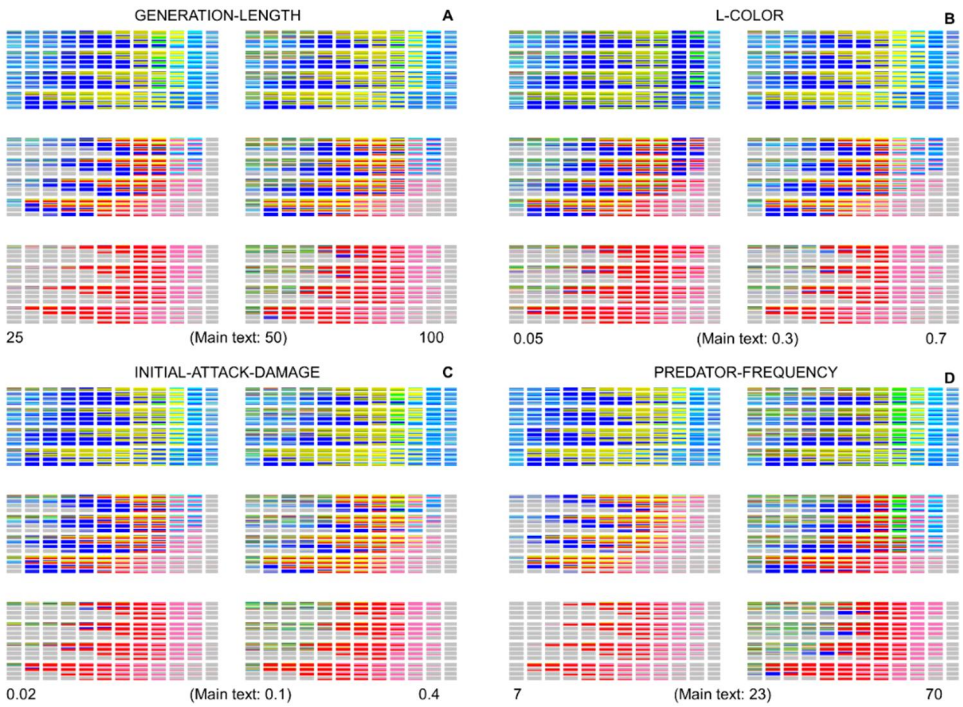
Supplementary Figure 4.1. The model result meta-plots with startle and learning facilitation effects.

(A) Results of simulations with STARTLE-EFFECT 0.2, HABITUATION-SPEED 0.2, LEARNING-AID-EFFECT 0.2, along with all the other variables identical to the main text **Figure 4.2B**. (B) Reproduced main text **Figure 4.2B** for easier comparison. (a) An example of a condition where the startle and the learning-aid effect change the simulation outcome. Color code and mini-plot structure is given in main text **Figure 4.1**. Post-attack strong signals were more favored if there were startle and learning-aid effects. The difference was most noticeable in high **predator turnover**, low **basal detectability**, and low **learning speed**. This figure is given as an example of how the program *ApoSim* can be used to simulate effects that are not covered in the main text. Users are encouraged to run the simulation with different sets of variables to further understand the variability of aposematic signals.


repeated runs
signal penalty
predator turnover
basal detectability
learning speed
NNN
NNL
NNH
LLL
HHH
NLL
NHH
LLH
LHH

Supplementary Figure 4.2. The model result meta-plots represented in 2-dimensional view.

The contents of this figure is identical to the main text **Figure 4.3**; only the perspective is changed to provide an alternative visualization.



Supplementary Figure 4.3. Result of parameter-space bracketing.

(A) Generation length varied. (B) Low-signal ("L-COLOR") varied. (C) Initial attack damage varied. (D) Predator frequency varied. The simulation runs depicted in this figure are performed to demonstrate the general robustness of the main text result. Below each subpanel, variable levels used for both extreme cases are given at the leftmost and rightmost corners, in addition to the level used in the main text in the middle. These four variables were chosen for this analysis because they were expected to differentially impact the different signal strategies, and at the same time, because they were reasonably expected to vary considerably without altering the core nature of the aposematism-driven system.

ApoSim User Manual

(= Information Tab of the NetLogo model file)

WHAT IS IT?

This project models aposematism (anti-predatory warning signal), predator learning and memory, and prey evolutionary dynamics. This model's main strength lies in the modelling of switchable aposematic signals. Unlike permanently colored animals, some prey animals can manifest warning signals only when attacked. This ability to switch signals can provide unique advantages, because the highly detectable signal can be hidden when not necessary. The model's approach involves setting up cognitive and ecological characteristics of predators and determining the prey's evolutionarily stable ("winning") strategy(-ies), from among a user-defined set of prey signaling strategies in the specific ecological conditions defined by several types of costs of signaling.

HOW IT WORKS

The accompanying article (Song et al. submitted) explains the internal rules the prey / predator individuals follow in detail. The following paragraphs summarize the paper's Method section briefly, but it is strongly recommended to read the full paper instead of this information tab.

The prey individuals can have three states, resting, approached, or attacked. In the 'approached' state the prey can give pre-attack signal to the predator. In the 'attacked' state the prey can give post-attack signal. The prey signal intensity is expressed at three levels, none, low and high. Therefore, the prey behavioral strategy is modeled as a sequence of three input-output pairs like the following:

[(resting: None), (approached: Low), (attacked: High)]. The above sequence can be further summarized as three-character notation, "NLH." This gives the user the opportunity to define a variety of possible behavioral strategies in the RULES-LIST window. For example NNN indicates non-signaling prey, and LLL or HHH indicates a typical fixed aposematism at a lower or higher level of signal intensity, and NNH indicates post-attack displays of high intensity, and NHH indicates pre-attack displays of high intensity, etc. The behavioral strategy must have 3 letters, and otherwise the program will give an error.

Predators have two attack opportunities, 'initial attack' and 'final attack.' The prey's pre-attack signal is given before the initial attack, and it can subsequently influence the probability of later attacks. Likewise, the post-attack signal is given after the initial attack and before the final attack. The post-attack signal can subsequently influence the probability of the final attack.

The basic time unit of the simulation is the 'interaction frame.' Each frame includes three 'time steps.' The Step 1 is when the prey shows a pre-attack signal if a predator is present in the same grid. The Step 2 is when the initial attack occurs and when the post-attack signal is given. The Step 3 is when the final attack occurs.

Predators have 'aversive memory' that increases after initial- or final attack events. If a predator has high level of aversive memory, it has lower probability of attacking the prey. The speed of memory buildup and the efficacy of retrieval are affected by a number of factors, described in Song et al (submitted). The predator's ‘decision’ to attack or not affects the 'survival chance' of the prey, which in turn determines the prey’s success of reproduction.

In the reproduction stage, prey individuals differentially reproduce based on their survival chance and the existing generation dies out. At the same time, the predator

population is refreshed by the adjustable variable 'predator turnover.'

During the interaction, the prey may pay a variety of costs that lower its survival chance. First, there is a 'switching cost' that is paid every time the signal intensity changes. Second, there is a 'switchability maintenance cost' that is continuously being paid if the prey has the ability to change the signal intensity. Third, 'signal penalty' is a cost paid during the presentation of the signal. Finally, the 'initial attack damage' and the 'final attack damage' are costs that are applied when the predator performs respective actions.

From the prey's viewpoint, the main benefit of a stronger signal is that, combined with the adjustable variable ‘learning speed’, it amplifies the buildup and retrieval of the predators’ aversive memory.

The main drawback of a stronger signal is that it can betray the prey's position to predators. The 'basal detectability' variable governs how the prey detectability changes depending on the signal intensity.

The detailed algorithms of the outlined model mechanics are available in the paper (Song et al. submitted).

HOW TO USE IT

The model interface has three buttons, 'Play/Pause,' 'One Frame Forward,' and 'Apply Parameters and Generate World.' For the first-time users, it is recommended to click the 'Apply Parameters and Generate World,' wait for the world generation and then click the 'Play/Pause' button. This will help the users to become familiar with the workings of the model under the default parameter settings. It would be necessary to accelerate the model speed to actually visualize the evolutionary process.

The most important reporting interface is the 'Rules Histogram' plot. Relative abundance of each behavioral strategy is displayed here, and the strategies are sorted by the order specified in the RULES-LIST. In the model view, prey individuals are represented by 'butterfly' shapes, and the predators are depicted as yellow 'default' shapes. Depending on the signal intensity (none, low or high), the color of the prey changes from black to dark red to bright red.

The rest of the interface elements are outlined below.

Number of Rules monitor displays the number of behavioral strategies specified via RULES-LIST editing interface.

Generation monitor displays the number of prey generations that have passed.

Predators monitor displays the number of predators present.

Preys monitor displays the number of prey present.

Frames monitor displays the number of interaction frames that have passed.

Average Aversion monitor displays the current average value of the aversive memory of predators.

Time Step monitor displays the current time step of the frame, 1, 2, or 3.

Predator Aversion histogram displays the current distribution of predator aversive memory.

The label displayed next to each prey individual in the world view represents the behavioral strategy of that prey, according to the order listed in the RULES-LIST.

PLOT-ON toggle button is used to suspend plot updating when computation speed is preferred over visualization.

As the model currently has 19 parameters that can be adjusted by the sliders, users are encouraged to test them one by one. The three 'World Parameters' need re-generation of the model world to take effect, but the other 16 should immediately affect the model.

WORLD-SIZE

The size of the world. Theoretically no limits. The larger the world the slower is the program running speed, but the outcomes will have better reproducibility.

PREDATOR-FREQUENCY

Number of predators divided by the number of all patches. This may be viewed as a degree of saturation of the habitat with the predators, or as a proxy for density of predators.

PREY-FREQUENCY

Number of prey individuals divided by the number of all patches. This may be viewed as a degree of saturation of the habitat with the prey, or as a proxy for density of prey. Their relative density can play an important role in aposemtism evolution, as the encounter rate can determine whether the learning can be achieved with an acceptable risk(Sword 1999)

GENERATION-LENGTH

Number of interaction frames per prey generation. As the effect of GENERATION-LENGTH is generally proxied by LEARNING-SPEED (whether the predators can

completely learn during the lifetime), it is advisable to use this variable as a mean of computational optimization only (giving more time vs. a larger world)

BASAL-COGNITIVE-CUE

Signal-independent cue from prey to predators. It corresponds to prey phenotypic traits, except the signaling, that affect the predator learning and predator memory retrieval. If BASAL-COGNITIVE-CUE is 1, the predator learning and retrieval are not affected by signal intensity at all. If BASAL-COGNITIVE-CUE is 0, the predator learning and retrieval are entirely dependent on the signal (neither learning nor retrieval can occur if there is no signal). Predators are shown to learn to avoid non-conspicuous defended prey, albeit at the slower speed (Kang et al. 2016b). Therefore, it is important to model diverse predator-prey systems with different 'basal' learning speed using this variable.

L-COLOR

Signal intensity between 0 and 1 at the 'low' level signal.

H-COLOR

Signal intensity between 0 and 1 at the 'high' level signal.

BASAL-DETECTABILITY

The chance of prey being discovered when no signal is present. If BASAL-DETECTABILITY is 1, the prey is always discoverable regardless of the signal. If BASAL-DETECTABILITY is 0, the prey is invisible when no signal is given. This variable is analyzed in detail in the accompanying article (Song et al. submitted).

Users might be interested in other discussions related to the detectability of non-signaling prey (Broom et al. 2010; Willink et al. 2013; Umbers & Mappes 2015).

BAD-TASTE

The strength of the aversive unconditional stimulus felt by the predators. Currently this single parameter controls two separate mechanics. First, it determines the learning-independent motivation drop after the initial attack. When BAD-TASTE is 0, the predator motivation would be unaffected unless some other effects are present. If BAD-TASTE is 1, the predator motivation would drop to zero after initial attack regardless of any other effects.

Second, it affects the aversive memory acquisition. If BAD-TASTE is 0, the predator would not be able to learn any aversion. If BAD-TASTE is 1, the predator learning is faster (how fast it is would be determined by a number of other factors).

PREDATOR-TURNOVER

The portion of predator population that are replaced with naive individuals per each prey generation. This variable is analyzed in detail in the accompanying article (Song et al. submitted). Predator turnover is one of the most prominent mechanisms that can result in a mix of naïve and experienced/educated predators (Endler & Mappes 2004), which may lead to diverse effects in the evolution of aposematism.

LEARNING-SPEED

The main coefficient that largely determines the speed of aversive memory buildup. If LEARNING-SPEED is 0.0, no aversion learning can occur. If LEARNING-

SPEED is 1.0, then a single experience with a prey of BAD-TASTE 1.0 and signal intensity 1.0 will lead to 1.0 increase in aversive memory. LEARNING-SPEED does not have an upper bound, and an arbitrarily high value of LEARNING-SPEED will enable arbitrarily fast aversive memory buildup. For the detail of the processes regarding aversive memory and its implication in the evolution of switchable aposematism, reading the accompanying article (Song et al. 2019) is recommended. There are also pertinent empirical (Kang et al. 2016b) and theoretical (Speed 2001; Puurtinen & Kaitala 2006) discussions of this variable.

SIGNAL-PENALTY

The cost (drop in survival chance) unconditionally paid by the prey due to the signal intensity. This variable summarizes all types of signal cost except the detectability cost. If SIGNAL-PENALTY is 0.0, there is no inherent cost in high-intensity signal except that it can draw predators' attention. If SIGNAL-PENALTY is 1.0, displaying an intensity=1.0 signal will instantly drop its survival chance to zero. The accompanying article (Song et al. submitted) discusses this variable in more detail. The costs of signal other than detectability can have a variety of effects (Blount et al. 2009) on the evolution of aposematism, including interactions outside of this model's scope.

SWITCHING-COST

The cost (drop in survival chance) paid by the prey whenever its signal intensity changes. If SWITCHING-COST is 0.0, there is no cost in signal switching. If SWITCHING-COST is 1, altering from intensity=0.0 to intensity=1.0 or vice versa will instantly drop its survival chance to zero. Along with the SWITCHABILITY-

MAINTENANCE-COST below, this variable has been explored in the accompanying article (Song et al. submitted). A previous theory (Broom et al. 2010), while not directly related to switchable signals, provide a good perspective about the potential of such cost affecting aposematism evolution.

SWITCHABILITY-MAINTENANCE-COST

The cost (drop in survival chance) unconditionally paid by the prey if the prey has a potential to change signal intensity. If SWITCHABILITY-MAINTENANCE-COST is 0.0, prey that does not actually switch signals would pay no cost even if they have the ability to do so. If SWITCHABILITY-MAINTENANCE-COST is 1.0, prey that are capable of switching signals will suffer instant elimination of any survival chance, regardless whether such switching behavior actually occurred or not. Along with the SWITCHING-COST above, this variable has been explored in the accompanying article (Song et al. submitted). A previous theory (Broom et al. 2010), while not directly related to switchable signals, provide a good perspective about the potential of such costs to affect evolution of aposematism.

INITIAL-ATTACK-DAMAGE

The cost (drop in survival chance) paid by the prey each time a predator conducts 'initial attack (the attack before seeing post-attack signal)' on it. If INITIAL-ATTACK-DAMAGE is 0.0, there is no damage from the attack. If INITIAL-ATTACK-DAMAGE is 1.0, a single attack event eliminates all survival chance. The survival rate after being attacked has long been regarded as a key factor in aposematism evolution (Wiklund & Jarvi 1982). The difference of this variable from the FINAL-ATTACK-DAMAGE below can arise from predator response to

mimicry (Gamberale-Stille & Guilford 2004).

FINAL-ATTACK-DAMAGE

The cost (drop in survival chance) paid by the prey each time a predator conducts 'final attack (the attack after seeing post-attack signal)' on it. If FINAL-ATTACK-DAMAGE is 0.0, there is no damage from the attack. If FINAL-ATTACK-DAMAGE is 1.0, a single attack event eliminates all survival chance. The survival rate after being attacked has long been regarded as a key factor in aposematism evolution (Wiklund & Jarvi 1982). The difference of this variable from the INITIAL-ATTACK-DAMAGE above can arise from predator response to mimicry (Gamberale-Stille & Guilford 2004).

LEARNING-AID-EFFECT

Facilitation of aversive memory buildup due to change in signal intensity. If LEARNING-AID-EFFECT is 0.0, switched signals and permanent signals with the same intensity have same effect on predator learning. If LEARNING-AID-EFFECT is 1.0, a switched signal that had undergone intensity jump of 1.0 would have twice as great an effect as the equivalent permanent signal. This value has no upper bound, and infinitely high value of LEARNING-AID-EFFECT will have infinite advantage to switched signals compared to permanent signals. The effect of learning facilitation by switchable aposematism has been empirically tested (Kang et al. 2016b).

STARTLE-EFFECT

Drop of prey attractiveness (chance to decide to attack) due to switching of the

signal. If STARTLE-EFFECT is 0.0, signal switching itself does not decrease the attack frequency. If STARTLE-EFFECT is 1.0, prey that has changed the signal intensity by 1.0 would lose all attractiveness to predators that have seen such change for the first time. Note that the effect of startle can be attenuated by habituation as specified by the HABITUATION-SPEED variable below. The startle display (deimatism) and the warning signal (aposematism) often share critical components or are known to simultaneously govern predator psychology (Schlenoff 1985; Bates & Fenton 1990).

HABITUATION-SPEED

Speed of loss of effective STARTLE-EFFECT. The effective STARTLE-EFFECT approaches zero as the predator accumulates the observations of prey signal switching. If HABITUATION-SPEED is 0.0, the effective STARTLE-EFFECT never changes from the value specified with the interface slider. If HABITUATION-SPEED is 1.0, a single experience of jump of signal intensity by 1.0 will remove all effective STARTLE-EFFECT for the rest of the predator's lifetime. Different systems have been shown to have different habituation characteristics (Bates & Fenton 1990; Olofsson et al. 2012)

WHAT TO DO WITH IT

This model is built in NetLogo 5.3.1, which features a systematic parameter-sweeping tool called BehaviorSpace (https://ccl.northwestern.edu/netlogo/docs/behaviorspace.html). In order to explore a wide range of model parameters, the user may need to set up an ‘experiment’ as per the procedures outlined in the web manual. The accompanying article (Song et al.

submitted) is also centered on four BehaviorSpace experiments, which created the Figure 2A, 2B, 2C and the Supplementary Figure 1 of the paper, respectively. The user can use these BehaviorSpace experiments (via Tools->BehaviorSpace) as starting examples to design their own ones.

CHAPTER 5. GENERAL CONCLUSION

Behavior is a trait of an animal that enables choice between different options depending on the circumstances. Animals decide which food items to consume, which nesting site to settle, which way to flee from danger, which body parts to use, and even which of other behaviors to execute next.

As this dissertation presented, some animals show considerable flexibility in making such choices, while others have strictly defined courses of action that are not easily altered. This 'diversity of flexibility' can arise in every aspect of animal ecology and in every taxon. Insects are no different, and Chapters 2, 3 and 4 introduced flexible and permanent strategies found in collective foraging and aposematic behavior. It is interesting we can see a striking diversity of flexibility within a relatively homogeneous taxon; organisms that are subject to this study often have similar body structure and physiology, and many of them share diet. However, still they have considerable differences in terms of flexible and permanent strategies.

Flexibility, at first glance, seems desirable in most conditions. If chosen correctly, access to different toolkits should give substantial survival advantage in various environments, as shown in Chapter 2 and 4. However, as the presence of the permanent-strategy indicates, flexibility could come with its own cost. The animals need to correctly sense the environment and process the information in order to make the desirable decision. If such a system involves multiple individuals as we have seen in Chapter 3, such proximate mechanisms can be extremely complicated and obscure. This cognitive and sensory overhead could be quite expensive especially for small nervous systems such as those found in insects. For example, noctuid moths that can normally react to bat echolocation sounds, became

deaf relatively after relaxed of bat predation pressure (Fullard 1994).

Furthermore, flexible behavioral strategies sometimes require additional energetic cost every time the state change occurs; especially those animals with unkenreflex perform a major maneuver every time they attempt to change their primarily seen body color. In other cases, a special adaptation is required for enabling flexibility which can incur developmental and maintenance costs. In addition to organisms studied in this dissertation, another good example is the rattlesnake; snakes in general use permanent-silence strategy in terms of auditory signals and cues. Unlike other snakes, however, rattlesnakes and their close relatives evolved to use tail-beating sound as a flexibly controlled audio source. In order to obtain higher efficacy in this uncommon strategy, the rattlesnake made a costly adaptation; tail rattles. The rattle is a fragile structure that needs to be always lifted from the ground, and its presence prevents rattlesnakes from versatile tail-tip usage found in other snakes (Klauber & Klauber 1972).

Therefore, it is reasonable to expect that flexible-strategy animals might have interesting mechanisms to minimize the accompanying costs. Sound generation (Dowdy & Conner 2016) might be more affordable to species with vocal communication devices. Wing movement (Kang et al. 2016a) could be essentially free for all animals capable of flight. Chapter 3 of this dissertation showed how the eusocial machine of an ant colony is utilized to give flexibility in foraging. The conditions of evolution of flexible strategies, and the methodologies animals adopt to implement such strategies, are highly productive grounds for evolutionary and ecological research.

On the other hand, the commitment cost of the permanent strategy should also not be underestimated. Aposematic animals with increased exposure and the

risk of 'death spiral' in army ants are both prime examples of this (Mappes et al. 2005; Das 2017), and Chapters 2 and 4 of this dissertation demonstrated this idea in theoretical approaches. In order to be fully stabilized, permanent strategies should have clear and strong benefits that can outweigh the myriad problems naturally associated with being specialists. However, we still lack understanding of what benefit it might be for many of the permanent strategies, even if they have been popular research subjects for many decades.

In Chapter 2, I compared the two different foraging strategies that could be used by army ants in different food distributions. The radial raiding was advantageous to the directional counterpart when the food patches were of low quality, and the directional raiding performed better in the opposite condition. The radial raiding strategy was energetically superior in most of the tested conditions. On the other hand, directional raiding was favored if exhaustive exploitation of a unit coverage area is desired.

In Chapter 3, the behavior of carpenter ant (*Camponotus japonicus*) scouts and recruits were studied in the field. During the recruitment, the summoned foragers follow the scout's lead. Sometimes the recruits tracked the scout's past trajectory with very high fidelity (tight following), but it was not uncommon to see recruits advancing in loose, disorganized formations (loose following). Trajectory analysis revealed that the tight following increased the recruit's chance to correctly reach the food site to which the scout was leading. On the other hand, the loose following behavior increased the collective search coverage which might lead to better exploitation of scattered or mobile resources. I found that the scout's initial reaction to food would be correlated to the subsequent coherence of her recruits; if the scout herself showed explorative responses to the food offering, she may lead

others in a more explorative formation as well. After finding this, I attempted to see if the signal for coherence is given in the early stage of recruitment. After experimentally removing the scouts at the beginning of the recruitment, I concluded that their behavior is better explained by an early-notification model.

In Chapter 4, I studied my final topic, the switchable aposematism. I named this phenomenon and established the first theoretical background about its evolution. The 'switching' of aposematic signal may occur in reaction to a predator's approach (pre-attack) or attack (post-attack), and these two types had to be treated separately, as they sometimes had wildly different conditions for evolutionary stabilization, especially in terms of switching cost, basal detectability. Switchable signals in common required certain moderate levels of predator learning speed and relatively high, but not prohibitive, signal cost.

These studies produced new knowledge that can update our perspective into the evolution of behavioral flexibility, and its ecological manifestations. However, in retrospect, I diagnose many limitations and drawbacks that should be remedied with future studies with better scientific rigor. A common weakness that is consistently found in all chapters is the general overlook of hostile interactions that could have shaped the current behaviors of study subjects. In Chapter 2, the need to overcome prey defense could be, at least in the beginning of the army ant syndrome, an important evolutionary driving force. By removing this aspect from the model, I obtained more conservative and robust results, but the applicability of the conclusion became limited. In Chapter 3, I treated the presence of the competitors as a mere observed variation of the test environment. As it should have been easily studied by a set of manipulative, controlled experiments, I missed a prime opportunity to test an attractive hypothesis. In Chapter 4, I disregarded the

actual amount of harm a defended prey might cause after (attempted) consumption. In order to keep the model structure simple and save the computational powers, I decided to disable evolution of the predatory guild and greatly simplified their learning algorithm. It would have opened a fruitful sub-discipline if I had explored the alteration in prey defense and that in signal together.

As agonistic interactions involve one of the most fast-paced situational changes among all experiences in an organisms' lifetime, a quest to behavioral flexibility would greatly benefit from the future studies directed into such questions. This hindsight and specific limitations and remedies discussed in each chapter would be the basis of my subsequent works in this field, and I expect that the continued effort in this direction will contribute to our power of harnessing the insects' ability in agriculture, medicine, and industry.

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국 문 초 록

이 논문은 곤충 행동학상의 두 가지 주제를 중심으로 하여 유연성에 대한 이론적 및 실험적 연구들을 제시한다. 첫 번째 주제는 개미의 집단적 섭식 전략이다. 이 논문에서는 먹이찾기 행동에서 매우 낮은 행동적 유연성을 보이는 예시로 군대개미를 선택하였고, 매우 높은 유연성의 기전을 연구할 대상으로는 왕개미를 택하였다. 두 번째 주제는 방어능력을 갖춘 피식동물이 포식자에게 정보를 주어 그 부적합성을 알게 하는 현상, 즉 경고신호 (aposematism) 의 행동적 통제이다.

첫 번째 연구에서는 군대개미의 특화된 섭식 행동이 진화할 수 있게 한 이론적인 이점들을 조사하였다. 신열대구 군대개미 (Ecitoninae 아과) 의 집락은 일반적으로 100 여 미터에 이르는 가느다란 행렬을 특정 방향으로 지향하여 영소 (bivouac) 주변의 광활한 지역을 강습하는 것으로 알려져 있다. 이러한 강습 행동이 매번 다른 방향으로 12-17 번 반복된 후 집락은 새로운 지역으로 이주한다. 이와 같은 먹이찾기 방법에 대해 하나의 가설적인 대안은 방사적, 대칭적인 형태로 강습하여 수색대의 최전선이 점차 확장되는 원형을 형성하도록 전진하는 것이다. 이와 같은 두 가지 먹이찾기 방법을 다양한 먹이자원 분포 하에서 비교해보기 위해, 군대개미의 행동을 시뮬레이션하는 기존의 개체 기반 모델링 소프트웨어를 활용하였다. 먹이패치의 질이 낮은 경우, 먹이패치의 풍부도와 무관하게 방사형 강습 행동은 지향성 강습보다 우월한 결과를 보였으며, 반면 먹이패치의 질이 높은 경우 지향성 강습이 유리하였다. 에너지 효율의 측면에서는 광범위한 조건에 걸쳐

방사형 강습이 더 나은 전략인 것으로 밝혀졌다. 그에 반해, 지향성 강습은 탐색 면적 당 더 많은 먹이를 구할 수 있는 경향이 있었다. 이 연구에서는 이 모델을 기반으로 하여, 군대개미의 지향성 강습 행동이 양질의 먹이패치가 풍부한 서식지 환경에 적응한 결과임을 주장하였다. 이는 지금까지 누적된 실험적 측정값 및 기술적 모델들과 일치하면서 군대개미 행동군 (behavioral syndrome) 의 적응적 가치를 보여주는 최초의 이론적 논증이다. 이러한 결론은 군대개미와 그 서식지에 대해 기존에 알려진 생태학적 지식과 잘 일치하는 것이다.

두 번째 연구에서는 왕개미 (*Camponotus japonicus*)의 야생 집락을 대상으로 야외 실험을 진행하였다. 응집된 행진 대열만을 절대적으로 고수하는 군대개미와 달리, *C. japonicus* 는 집단적으로 먹이를 찾을 때 응집도의 변산이 상당히 크다. 이 연구에서는 다음의 세 가지 하위 질문을 중심으로 이 현상에 대한 조사를 진행하였다. 첫째, 집단의 분산도가 높으면 먹이원에 이르는 성공률이 낮아지게 되는가를 관찰하였다. 분산된 집단은 전체적으로 보았을 때 더 넓은 면적을 탐색하게 된다는 점을 감안할 때, 그에 대응되는 단점이 존재하여야 응집형 및 분산형 집단이 공존하는 현상을 설명할 수 있으리라 판단하였다. 둘째, 처음으로 먹이원을 발견하고 이후에 다수의 동료들을 불러모으는 중심 개체인 정찰병의 행동이 집단적 응집도와 어떠한 연관성을 지니는지 탐색하였다. 근연종의 정찰병들이 소집된 집단에 대해 다양한 방식으로 통제력을 행사하는 것이 알려져 있었으므로, *C. japonicus* 정찰병 역시 집단 행동의 결정에 관여할 것이라는 가설을

수립하였다. 셋째, 집단으로부터 정찰병을 제거하는 경우 어떤 반응이 나타나는지 시험하였다. 정찰병이 응집 신호의 근원인 것으로 생각되었으므로, 정찰병 페로몬의 부재는 곧 분산 신호로 해석될 것이라 판단하였다. 분석 결과, 이 연구는 다음과 같은 결론들에 도달하였다. 첫째, 집단의 분산도가 높으면 먹이원을 올바르게 찾아내는 성공률이 낮아진다. 둘째, 소집 전단계에서 정찰병의 이동성, 소집 중 정찰병의 '획긋기' 행동, 소집된 집단의 분산도는 모두 서로 연관되어 있다. 셋째, 정찰병 신호의 단순 부재만으로는 남겨진 추종자들의 다양한 반응을 모두 설명할 수 없으며, 이들의 반응은 소집 전단계에서 정찰병이 보였던 행동과 연결되어 있다. *C. japonicus* 는 개미과를 통틀어 가장 복잡한 소집전략을 지닌 종 중 하나로 생각되는데, 위와 같은 발견들을 통해 집단적 섭식행동의 유연성 및 그 제어방법이라는 영역에서 이 종에 대한 이해도는 개미 중 최고 수준에 이르게 되었다.

위의 두 연구에서는 개미의 섭식 전략에서 다양한 수준의 유연성이 유지되게 하는 궁극적 및 직접적 기전 한 가지씩을 규명하였다. 이 논문의 마지막 주제는 행동적으로 통제 가능한 경고신호, 즉 방어능력을 갖춘 피식동물이 상황에 따라 다른 수준의 반포식 경고신호를 택할 수 있는 현상에 대한 것이다. 이와 같은 신호의 '전환'은 포식자의 접근에 대응하여 발생할 수도 있고 (공격전 신호) 또는 공격에 대응하는 것일 수도 있다 (공격후 신호). 전환가능한 경고신호는 비교적 연구가 잘 되어 있지 않으나, 다양한 이점을 지닐 것으로 보인다. 첫째, 전환 행동 자체가 포식자를 놀라게 할 수 있다. 둘째, 회피 학습을

촉진할 수 있다. 셋째, 신호를 꺼 놓음으로써 불필요한 노출이나 에너지 소모를 최소화할 수 있다. 이와 같은 잠재적인 이점들은 전환형 형질을 발달시키고 유지하는 데 필요한 비용을 상쇄할 가능성이 있다. 이 연구에서는 전환가능성의 세 번째 이점, 즉 비용 절감 측면에 초점을 맞추어 포식자와 피식자에 대한 개체 기반 컴퓨터 시뮬레이션을 개발하였다. 88,128 회의 모델 구동을 통하여 다양한 강도의 영구적, 공격전 및 공격후 경고신호가 진화하는 과정을 관찰하였다. 일반적으로, 공격전 경고신호는 중등도의 포식자 학습속도, 높은 기저 발견률, 중등도에서 고도의 신호비용을 요구하리라는 점을 발견하였다. 그에 반해 공격후 진호는 낮은 포식자 학습속도, 낮은 기저 발견률, 높은 신호비용 하에서 나타날 가능성이 높았다. 포식자 개체군의 회전이 빠른 경우, 위의 경향성을 벗어난 공격후 신호의 진화가 가능하였다. 또한 이 연구는 높은 전환비용이 공격전 및 공격후 신호 전략에 대해 서로 다른 압력을 가하리라는 예측을 제시하였다. 이와 같은 발견들은 방어능력이 있는 피식동물에서 영구적 경고신호에 대해 전환가능한 경고신호가 진화하는 과정을 체계적으로 탐색하고자 한 최초의 이론적 시도이다.

이와 같은 발견들은 공통적으로 곤충에서 나타나는 행동적 유연성의 직접적 및 궁극적 기전에 대해 새로운 시각을 제공하였다. 또한 이 연구들은 후속 연구를 필요로 하는 이해의 한계를 노출하기도 하였다. 첫째, 경쟁자나 피식자와의 적대적 상호작용이 집단섭식, 경고신호 및 회피학습에 상당히 큰 영향을 끼칠 것으로 예상되나, 많은 노력에도 불구하고 현재 가용한 데이터와 문헌으로는 이같은 효과에

대해 범용적인 배경지식을 생산할 수 없었다. 둘째, 탄수화물 또는 단백질 함량과 같은 먹이자원의 질적 차이가 곤충의 영양생태에 중대한 영향을 준다는 것이 알려져 있으나, 정보의 희박함과 자원의 한계로 인하여 이와 관련된 조사를 본 논문에 포함시키지 못하였다. 이러한 질문들에 대한 추가적인 논의와 실험이 이루어진다면, 생태계에서 관찰되는 곤충의 행동적 유연성에 큰 영향을 주고 있을 자원 획득과 방어의 균형에 관한 가치 있는 정보들이 밝혀지리라 예상한다.

주요어 : 개미, 섭식, 대열, 정찰병, 소집, 경고신호, 전환가능성

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Some people seem to be actually islands, entire of themselves; but at least I am certainly not. Surrounding me is never an ocean's barrenness but the charming forest of wisdom, meadows of love, and loud waters of hope winding through steadfast hills of trust. I have never wanted to write a dissertation, but I have carried on for no other reason than this opportunity to finally write about the beautiful landscape wherein my soul resides.

I first met Professor Piotr G. Jabłonski with bulky binoculars suspended on his shoulders in a field jacket – in the middle of a seminar room full of suit-clad academics, completely encircled by a windowless wall, a hallway of marble slabs, another wall, concrete, bricks, and then a strip of asphalt. Enthusiasm was not a word that did proper justice for that. It was almost an aposematism – I think it actually worked quite well to deter certain types of society that suffocate scientists. Into that safety bubble I was naturally attracted, and this is the life I ended up in. He is an amazing instructor, mentor, colleague, and supervisor, and I could have easily written a book full of thankful episodes of which a graduate student from another lab would not believe even a single story. But instead of that, I decided to just say that what he is really like; I believe that this is actually how he wants to be acknowledged and remembered.

Professor Sang-im Lee is demonstrating that one person can know everything, feel everyone, be everywhere, and still at the same time be strong and free. I still have no idea how she does any one of that, not to speak all of them combined. If a student's job is to grow to be someone like the educator (and ideally better than her), I continue to hopelessly fail her course after 10 years of effort and development. I am not alone in this party; many of our students openly dream to

speak like her, write like her, think, see, and act like her. In a child's eyes, the night sky is full of light speckles that seem unreachably high. After some years, the child learns that some of them are airplanes, not the celestial bodies. Yet after some other years, the kid becomes a spacecraft pilot and flies higher than the planes. One day the kid visits the orbits of Venus and Jupiter, and he realizes that he really is voyaging the very same realm of planets as his childhood remembers. But still, there is a dot of light never gets closer to him; a star, many light-years far, is still at the same position, unreachably and unimaginably high. She, is that star.

One of my secrets is that I rarely read textbooks. I pretend I read them, but my motivation generally does not last that long. However hard I (and the instructors) try, I have taken many dozens of final exams after only cherry-picking and skimming over the book. Nevertheless, I have actually gone through the full textbook, not once but twice, for one course in my life. This lecture was taught by Professor Eunju Lee, my committee chair. His quiet yet powerful energy of trust and optimism has done another wonder to me during the preparation of my defense.

Science should be understood; this one sentence summarizes the lessons I have learned from Professor Ikwon Jang for the last seven years. As an expert in citizen science, he is capable of making whole-conference-worth ideas deliverable to seven-year-olds. I had an opportunity to formulate the presentation of my study under his guidance, a rare prestige. My work was initially obscure to even myself, but thanks to him I was capable of forming a story of discovery.

A proud alumnus of our lab, Professor Choongwon Jeong, is a talented writer and quantitative analyst. Though his true skills cannot be imitated, my work has been greatly improved by merely heeding his styles and examples. As he is a young scientist of my generation, his counsel was a gift other committee members

cannot give. Thanks to his advice my dissertation formed an acceptable structure as scientific writing.

I would like to express my gratitude towards my best friends, the members of our lab. First and foremost, I am obliged to pay my tribute to the very special colleagues who walked through this year together with me.

The world has seldom seen a leader like Keesan Lee. If she preferred working with marines instead of passerines, I believe that history would have recorded a legendary commander whose unit can willingly march into the crucible of war. She is a nationally recognized student ornithologist and an inventor of many methodologies widely adopted in our lab. However, her true strength reaches far beyond her endeavor in science. She is an organizer, motivator, educator, cultivator, critic and a counselor all at the same time: cold steel, blazing flame, endless ocean and warm hearth combined in one soul. Thanks to her I have stayed detached from my personal rise and fall, and from myself. Through her eyes, I could see where and what I really am: a small piece of a grander scheme, a thread of a tapestry, a stone in a mountain. She made me step out of my small self and view the greater world, and I became capable of helping other people rather than diminishing as an isolated being.

Initially, I attempted to mentor Hangah Lim, but soon I found myself mentored by her. She knows how to live under the burden of reality, and in hard times her strengths truly shine. Her ingenuity, resilience, adaptability, and above all her fighting spirit has maintained her as an inspiring and helping figure. It is a wonder beyond belief to behold in eyes of people like me, who become discouraging and dependent under prolonged hardships. After half a year of

working with her, I developed a habit of asking myself, “how would she do?” in times of pain and difficulty. For the rest of my life, this question and the vivid memory of such great power will remain as my guiding light in the deepest of nights.

If I am given an opportunity to design a better version of myself, it is doubtless I would envision someone like Injae Hwang as the model. She shares similar weaknesses to me, but overcomes them more wisely and bravely; similar strengths to me she also has, but she wields them with more skill and care. Preparing a thesis is a series of problem-solving after one another, and in every step I could learn from her adept solutions. She is a natural-born diplomat not just between people, but between skills, cultures, worlds, and bodies of knowledge. We ‘Thesis Preppers’ naturally have formed a team of a sort, and it has been she who makes the team greater than the sum of individuals.

I also thank all of our lab members who has supported my life in this little haven. Dr. Hyun-young Nam, an ornithologist with specialties in migration and sexual selection, has provided valuable counsel to us from her experience as a senior researcher. Jihoon Lee, the one with whom everyone wants to work together in both science and administration, is maintaining the strong foundation and logistics that I and many others depend on. Yeojoo Yoon, the leading student of the magpie team and the sequence analysis specialist, never reserved a help when I or anyone was in need. Haeun Cho, a prolific writer and energetic bird enthusiast, is beginning to take on the most demanding jobs of both field and molecular works. Jihyeon Song, a symbol of perseverance and self-taught greatness, is beginning her career in frass analysis in addition to her birdwatching expertise. Jeongmoon Ha, the craftsperson, international organizer and bird-sound specialist, is serving as the

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they could not risk a failure; and only thanks to them, I could afford many failures and still be left with chances to try again.

I dedicate this work to the late Sgt. 1st Class Byungsun Park, my grandfather, who gave me my first field guide when I was a toddler. It was an old, sun-bleached book partially rescribed with his handwriting. When the day comes I meet him again, I believe he must have finished a new guide to the heavenly ecosystem for me.